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STUDIES

FROM THE

LABORATORY OF PHYSIOLOGICAL CHEMISTRY

SHEFFIELD SCIENTIFIC SCHOOL

OF

YALE UNIVERSITY

For the Year 1885-86

VOLUME II

EDITED BY

R. H. CHITTENDEN, Ph.D.
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[From Transactions Connecticut Academy, Vol. VII, March, 1887.]

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Globulin and Globulose.*

By W. Kühne, Professor of Physiology in the University of Heidelberg, and R. H. Chittenden.

The following investigation is a continuation of our previous work with the albumose bodies,† and constitutes the commencement of a study of the various primary cleavage products, formed by the action of pepsin from the better characterized and purer albumins. Our work in this direction was at first begun with the albuminous bodies occurring in blood serum, egg albumin, and in fibrin and from the results then obtained we were led to the conviction‡ that an extension of the work to the single albumins was necessary, particularly to serum albumin, globulin and myosin. In the meantime, our first conjecture that hemialbumose might possibly be a mixture, had been established and led finally to the isolation of the albumose bodies of fibrin.§ At this stage of our work, fibrin was the only albuminous material employed because it was the simplest to prepare, was easily digestible, and hitherto had been mostly used in digestion experiments. These practical considerations, to which must be added the fact that fibrin albumose was later found as a commercial article, were joined with the advantage of being able to make use of, and at the same time extend, the discoveries in the chemistry of digestion which have for decades been made with this particular albuminous material. Other advantages for the use of fibrin in this connection cannot well be claimed, since its peculiar position between the genuine and the coagulated albumins, its contamination with globulin and the presence of nuclein and other components of the white blood corpuscles tend to render it perhaps least fitted for use in an exact study of albumin cleavage; and since fibrin is of importance as a food stuff mainly in the form of the commercial so-called peptone, there are many reasons for proceeding to the study of other digestible substances for further results.

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* This and the following article were originally published in the Zeitschrift für Biologie, Band xxii. They are republished here to render the series complete in English.

† See Amer. Chem. Jour., vol. vi, p. 3.

‡ Zeitschrift für Biologie, Band xix, p. 159.

§ Ibid., xx, p. 11.

As we hope to show, however, the results gained in the study of the albumose bodies from fibrin are by no means to be considered as valueless, but on the other hand as quite advantageous in the treatment of the single albumins, particularly as an aid in the separation of the individual albumose bodies. Thus in our study of globulin, now to be described, we were able to use methods already tested.

The new method of precipitating albumose bodies by ammonium sulphate, which has meanwhile been discovered and which is certainly particularly advantageous, has not been used in our study of the globulose bodies, since these experiments were concluded before the introduction of that method. According to observations made by Dr. Neumeister in the Physiological Institute at Heidelberg, ammonium sulphate appears particularly well adapted to the purification of deuteroalbumose, since after complete removal of protoalbumose by sodium chloride and acid, deuteroalbumose alone is precipitated by addition of the ammonium salt. As, however, we had accomplished a separation of the different globulose bodies in another manner, it seemed unnecessary to use, in addition, the new method.

Globulin.

From the list of albuminous bodies which occur in sufficient quantity, and which at the same time admit of isolation without too great difficulty we have chosen for our first study, globulin. The substance was prepared from the serum of ox blood by the method of Hammarsten, in which repeated quantities of fresh serum were treated with an excess of crystallized magnesium sulphate at a temperature of 30° C. The precipitate so obtained was collected on a filter, washed with a saturated solution of the salt, pressed, dissolved in water, and reprecipitated a second time by the addition of magnesium sulphate. The precipitate was then dissolved in water, a little thymol added, and the solution dialyzed in running water in order to remove the magnesium salt. The final solution was then concentrated at 40° C., after which the globulin was precipitated with alcohol, washed with alcohol and finally extracted with ether. The substance so prepared, and which still contained some magnesium sulphate, was employed in the digestion experiments. A portion of the preparation, dried at 110° C. *in vacuo*, gave by analysis the results contained in the following table. The methods of analysis were the same as those employed in our previous work.*

* Compare Zeitschrift für Biologie, Band xx. p. 11, and Amer. Chem. Jour., vol. vi, p. 3.

Analysis of Globulin.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of ash. % of substance.	$BaSO_4$ after fusion with KOH + KNO_3 gram.	S %	S after deducting S of ash. %
I	0·6310	0·3833	6·74	1·1425	49·37	....	..	....	....	....	....	....	....	....	....	....
II	0·5122	0·3120	6·76	0·9270	49·35	....	..	....	....	....	....	....	....	....	....	....
III	0·8265	....	....	....	....	94·63	4·6	761·6	14·17	....	....	....	....	....	....	....
IV	0·5773	....	....	....	....	64·85	5·5	773·8	14·08	....	....	....	....	....	....	....
V	1·4916	....	....	....	....	....	..	....	....	0·0520	3·49	0·1010	0·98	....	....	....
VI	1·1170	....	....	....	....	....	..	....	....	0·0387	3·47	0·0756	0·92	....	....	....
VII	0·8445	....	....	....	....	....	..	....	....	....	....	....	....	0·1543	2·50	1·57
VIII	0·5032	....	....	....	....	....	..	....	....	....	....	....	....	0·0919	2·50	1·58

Percentage composition of ash-free Globulin.

							Average.
C	51·15	51·13	....	....	....	....	*51·14*
H	6·99	7·01	....	....	....	....	*7·00*
N	....	....	14·68	14·59	....	....	*14·64*
S	....	....	....	....	1·66	1·67	*1·67*
O	....	....	....	....	....	....	*25·55*
							100·00

Digestion of Globulin.

The preparation proved to be extremely difficult of digestion; this fact, however, enabled us to remove from the globulin the magnesium sulphate still remaining, since the large quantity of dilute hydrochloric acid added with the small amount of pepsin, failed at first to dissolve any of the albuminous matter. The undigested residue was then finally treated with an artificial gastric juice much richer in pepsin. 250 grams of the powdered globulin were at first warmed with 5 litres of 0·2 per cent. hydrochloric acid for 24 hours, whereby the substance swelled up to double its former bulk, but only a trace was dissolved; barely enough for a filtered portion to give with nitric acid and heat a slight turbidity. By the addition of 200 c. c. of normal gastric juice* to the swollen mass and warming it at 40° C. for 24 hours longer, the albuminous matter was not appreciably changed; a filtered portion, however, became turbid on neutralization and a small amount of albumose could be detected both with sodium chloride and with nitric acid. The entire mass of swollen globulin was then collected on a cloth filter, washed thoroughly with 0·2 per cent. hydrochloric acid and warmed again at 40° C. for six days with 4 litres of a particularly active gastric juice, containing 0·4 per cent. hydrochloric acid and 0·45 per cent. of solid matter. By this last treatment, a large amount of globulin was dissolved. As the digestive mixture would not filter through cloth it had to be neutralized directly with sodium hydroxide, which gave an abundant neutralization precipitate of so-called parapeptone, easily collected, and from which the fluid filtered perfectly clear. In order to obtain more material for study, the neutralization precipitate, together with the unaltered globulin, was treated a second time with 3 litres of the same active gastric juice for several days, by which the amount of globulin was reduced more than half, as shown by repeated neutralization.

The two neutralized, digestive fluids obtained in this manner, were alike in all respects; noticeably so in the remarkable fact that when weakly alkaline, they became turbid at 53° C., which turbidity increased as the solutions were heated to boiling. Furthermore, when made faintly acid, heat produced in both solutions an abundant flocculent precipitate which had the properties of coagulated albumin. This *coagulum from digested globulin* was purified by successive washings with boiling water, alcohol and ether. The following table shows the composition of the substance.

* See Zeitschrift für Biologie, Band xix, p. 184.

ANALYSIS OF THE COAGULUM FROM DIGESTED GLOBULIN.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
I	0·7229	0·4443	6·82	1·3667	51·55	----	----	----	----	-----	----	-----	----
II	0·4744	0·2950	6·90	0·8971	51·56	----	----	----	----	-----	----	-----	----
III	0·5634	-----	----	-----	----	75.0	18·8	765·2	15·75	-----	----	-----	----
IV	0·3546	-----	----	-----	----	47·43	19·6	764·2	15·76	-----	----	-----	----
V	0·6072	-----	----	-----	----	----	----	----	----	0·0753	1·70	-----	----
VI	0·6474	-----	----	-----	----	----	----	----	----	0·0893	1·89	-----	----
VII	0·7602	-----	----	-----	----	----	----	----	----	-----	----	0·0073	0·96
VIII	0·6724	-----	----	-----	----	----	----	----	----	-----	----	0·0060	0·89

The ash consisted mainly of Fe_2O_3.

Percentage composition of ash-free substance.

							Average.
C	52·03	52·04	----	----	----	----	*52·03*
H	6·89	6·97	----	----	----	----	*6·93*
N	----	----	15·89	15·90	----	----	*15·89*
S	----	----	----	----	1·71	1·90	*1·80*
O	----	----	----	----	----	----	*23·35*
							100·00

Globulose Bodies.

After removal of the above mentioned coagulum, the solution remained perfectly clear at all temperatures up to 100° C., even when rendered more strongly acid and also on subsequent neutralization. On the addition of nitric acid, however, to the cold solution, a precipitate was formed which disappeared on the application of heat, reappearing as the mixture became cool.

Crystals of salt alone, produced a heavy precipitate in the solution, while salt and acetic acid gave a still further precipitate, and when these reagents failed to cause any further precipitation, nitric acid or metaphosphoric acid would still give a noticeable turbidity.

In order to separate the globulose bodies from one another, the entire solution was concentrated on a water bath to the consistency of a thin syrup and then rubbed up in a mortar with salt in substance (the fluid being perfectly neutral), complete saturation being insured by long standing with an excess of salt crystals. The precipitate so formed being separated by filtration, the filtrate was partially precipitated by the cautious addition of 30 per cent. acetic acid saturated with salt, whereby a mixture of proto- and deuteroglobulose was separated. After removal of this precipitate the filtrate was finally treated with more of the above acetic acid until nothing further was precipitated.

The various precipitates were then subjected to strong pressure to remove as much of the salt-saturated fluid as possible, then dissolved in water and dialyzed for the complete removal of the salt and to separate heteroglobulose.

Protoglobulose.

This body, precipitable by sodium chloride alone, was purified by saturating the first dialyzed solution again with salt, then dialyzing a second time under repeated changes of reaction, by the alternate addition of acetic acid and sodium carbonate and finally by neutral reaction, until all chlorine was removed from the solution and the admixed heteroglobulose completely separated. The clear filtered fluid was then concentrated, after which the protoglobulose was precipitated with alcohol, washed with alcohol and ether and so obtained as an almost white powder. The substance, so prepared, gave when rubbed up with cold water, a filtrate not quite clear and with a noticeably alkaline reaction. It differed from a solution of protoalbumose from fibrin in one respect, viz: that on boiling in the presence of a small amount of sodium chloride it became quite turbid,

the turbidity, however, disappearing completely as the solution became cool, (the opposite of the albumose reaction : compare Zeitschrift für Biologie, Band xx, p. 45). We think it may be assumed that this single deviation from the reactions of fibrin-protoalbumose is not due to the presence of impurities (heteroglobulose, etc.), because no heteroglobulose whatever separated from a portion of the sample, even after dialyzing a week longer. If the solution was made even very slightly acid or alkaline, the turbidity did not then occur on heating. Further, the small content of ash (0·4 per cent.), which consisted only of calcium sulphate with a trace of ferric oxide, testifies to the purity of the preparation and the completeness of the dialysis. The preparation was analyzed with the results shown in the accompanying table.

Deuteroglobulose.

This body is about as difficult to purify from the preceding one as deuteroalbumose from protoalbumose. We succeeded, however, in separating it by rejecting the first portions of the precipitate produced by acetic acid and sodium chloride and using only the last portions precipitated; or after the acetic acid failed to give any further precipitate, by using the small precipitate produced by the moderate addition of alcohol. This last precipitate naturally enclosed considerable sodium chloride, but the deuteroglobulose was obtained perfectly pure after removing the salt by dialysis, since the globulose solution, even when noticeably acid, gave no turbidity whatever on the addition of salt in substance. The quantity, however, was unfortunately too small for analysis. It sufficed only for determining the reactions, which agreed with those of the substance obtained by the later precipitation with acetic acid except in one particular, viz: that the latter preparation in a neutral or slightly alkaline solution showed an extremely slight turbidity on the addition of crystals of sodium chloride. All other reactions were identical and corresponded so completely with those of deuteroalbumose that it is only necessary to call attention to the latter (see Zeitschrift für Biologie, Band xx, pp. 26–28, or Amer. Chem. Jour., vol. vi, pp. 46–47) and to especially mention the non-precipitation of deuteroglobulose in a solution free from salt, by nitric acid in any quantity and at any temperature.

Since no heteroglobulose whatever separated from the solution of the last acetic acid precipitate during dialysis, even with repeated change of reaction, the substance was therefore prepared for analysis,

ANALYSIS OF PROTOGLOBULOSE.

No.	Substance used. gram.	H_2O found. gram	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found gram.	Ash %
I	0·6707	0·4211	6·97	1·2577	51·13	....	....	....	....	....	....	....	....
II	0·4926	0·3070	6·92	0·9300	51·48	....	....	....	....	....	....	....	....
III	0·5328	....	....	....	....	72·3	18·8	763·56	16·02	....	....	....	....
IV	0·4313	....	....	....	....	59·23	19·9	758·14	16·03	....	....	....	....
V	0·7436	....	....	....	....	....	....	....	....	0·1188	2·19	....	....
VI	0·5765	....	....	....	....	....	....	....	....	0·0924	2·20	....	....
VII	0·9137	....	....	....	....	....	....	....	....	....	....	0·0043	0·47
VIII	0·5124	....	....	....	....	....	....	....	....	....	....	0·0025	0·48
IX	0·3350	0·2100	6·96	0·6308	51·34	....	....	....	....	....	....	....	....

Percentage composition of ash-free Protoglobulose.

								Average.
C	51·38	51·78	51·59	....	....	....	....	*51·57*
H	7·00	6·95	6·99	....	....	....	....	*6·98*
N	....	....	....	16·09	16·10	....	....	*16·09*
S	....	....	....	....	....	2·20	2·21	*2·20*
O	....	....	....	....	....	....	....	*23·16*
								100·00

simply by concentration of the solution freed from chlorine, precipitation with alcohol and washing with ether. The ash (1·17 per cent.) consisted only of calcium phosphate and a trace of sulphate.

Heteroglobulose.

This body was obtained from the gummy precipitate which separated, during dialysis, from the solution of the first precipitate thrown down from the neutralized digestive fluid by salt alone. The sticky mass was separated from the sides of the parchment tubes, dissolved in sodium chloride of from 3 to 5 per cent., reprecipitated by saturation of the solution with salt, the precipitate again dissolved in dilute salt solution and the substance finally separated by long continued dialysis in running water. After thorough washing with water, alcohol and ether it appeared as a light, white powder, not unlike heteroalbumose in general behavior and reactions. After each precipitation and treatment with dilute sodium chloride, heteroglobulose left a residue, which like dysalbumose was readily soluble only in dilute acids. From the following analysis it is to be seen that the preparation, in spite of its long continued and repeated dialysis, contained 2·03 per cent. of ash, which consisted mainly of calcium carbonate with a small amount of phosphate and sulphate.

The composition of the three globulose bodies shows the same slight differences as noticed in the case of the various albumose bodies (from fibrin). Unlike the latter, however, the content of carbon in the globulose bodies never falls below 51 per cent., and furthermore it is always higher than that of the globulin from which the globulose was derived. The percentage of nitrogen, which in the albumose bodies was found a little higher than in fibrin, exceeds that of the globulin more yet, in some cases by more than 1 per cent., and the same holds true of the percentage of sulphur. In contrast to the albumose bodies, the percentage composition of the globulose bodies gives no grounds whatever for the assumption that they arise from the digested globulin by simple hydration. It must not be forgotten, however, that the digestion of globulin by gastric juice is a process quite different from that of fibrin digestion and one hitherto much less clearly understood, since besides the globulose bodies there is formed a large quantity of a substance which is separated by boiling and which resembles ordinary coagulated albumin. Something similar, indeed, has been known ever since Brücke's study of fibrin digestion, but it has long been accepted that the coagulum

ANALYSIS OF DEUTEROGLOBULOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·4711	0·2910	6·86	0·8790	50·88	....	....	....	....	....	....	....	....
II	0·3468	0·2149	6·88	0·6481	50·96	....	....	....	....	....	....	....	....
III	0·5403	....	....	....	....	72·94	19·6	756·10	15·73	....	....	....	....
IV	0·5106	....	....	....	....	69·01	19·4	756·10	15·76	....	....	....	....
V	0·4616	....	....	....	....	....	....	....	....	0·0656	1·95	....	....
VI	0·5634	....	....	....	....	....	....	....	....	0·0693	1·69	....	....
VII	0·6472	....	....	....	....	....	....	....	....	0·0893	1·89	....	....
VIII	0·6540	....	....	....	....	....	....	....	....	....	....	0·0076	1·16
IX	0·6447	....	....	....	....	....	....	....	....	....	....	0·0077	1·19

Percentage composition of ash-free Deuteroglobulose.

								Average.
C	51·48	51·56	....	....	....	....	....	*51·52*
H	6·94	6·96	....	....	....	....	....	*6·95*
N	....	....	15·92	15·95	....	....	....	*15·94*
S	....	....	....	....	1·97	1·71	1·91	*1·86*
O	....	....	....	....	....	....	....	*23·73*
								100·00

ANALYSIS OF HETEROGLOBULOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
						c c.	T. ° C.	Pressure mm.					
I	0·5200	0·3187	6·80	0·9745	51·10	----	----	-----	----	-----	----	-----	----
II	0·3475	0·2149	6·87	0·6500	51·00	----	----	-----	----	-----	----	-----	----
III	0·3625	-----	----	-----	----	48·44	20·0	764·74	15·73	-----	----	-----	----
IV	0·3438	-----	----	-----	----	46·07	19·6	764·57	15·79	-----	----	-----	----
V	0·5227	-----	----	-----	----	----	----	-----	----	0·0822	2·15	-----	----
VI	0·6045	----	----	-----	----	----	----	-----	----	0·0916	2·08	-----	----
VII	0·5811	----	----	-----	----	----	----	-----	----	-----	----	0·0120	2·06
VIII	0·4897	----	----	-----	----	----	----	-----	----	-----	----	0·0098	2·00

Percentage composition of ash-free Heteroglobulose.

							Average.
C	52.15	52·05	----	----	----	----	*52·10*
H	6·95	7·02	----	----	----	----	*6·98*
N	----	----	16·05	16·11	----	----	*16·08*
S	----	----	----	----	2·20	2·13	*2.16*
O	----	----	----	----	----	----	*22·68*
							100·00

obtained by Brücke on boiling the neutralized digestive fluid, arose from the globulin present in the fibrin employed, which had not been previously washed with salt water. Globulin, moreover, yields this body in much greater quantity, even after several days' exposure to the action of an energetic gastric juice and it was still found abundantly among the products of a second digestion of the first neutralization precipitate.

Comparison of the Analyses.

	Globulin.	Coagulum from digested Globulin.	Proto-globulose.	Deutero-globulose.	Hetero-globulose.		Hemialbumose from urine* (Osteomalachia).	Hetero-albumose from Fibrin.†	Fibrin ‡
C	51·14	52·03	51·57	51·52	52·10	C	52·13	50·88	52·68
H	7·00	6·93	6·98	6·95	6 98	H	6·83	6·89	6·83
N	14·64	15·89	16·09	15·94	16·08	N	16·55	17 08	16·91
S	1·67	1·80	2·20	1·86	2·16	S	(1·09 ?)	1·23	1·10
O	25·55	23·35	23·16	23·73	22·68	O	23·40	23·92	22·48

In this review of the composition of globulin and of the products of its digestion we have included also an analysis of fibrin, of a fibrin-albumose and of hemialbumose from the urine of a person with osteomalachia.

We call attention again to the latter because its surprising correspondence, especially to heteroglobulose, appears to confirm the belief, expressed in our former paper that the difference in the albumose from urine and that from fibrin depends on the formation of the former from an albuminous body, whose digestion, at least as regards the formation of albumose bodies, was then unknown, and for which we had already turned to globulin.

In order to gain further information concerning the cleavage of globulin in the process of digestion, the remaining material was used in the following experiments.

1. Heteroglobulose dissolved in 0·3 per cent. sodium carbonate and warmed at 40° C. for fourteen days with pure trypsin (with the addition of thymol, as usual) remained perfectly clear, even after neutralization, and failed to yield afterwards any body resembling antialbumid. Among the products of the digestion, there was found in addition to an abundance of antipeptone, only a trace of leucin, no tyrosin whatever, while with bromine water the alcoholic extract,

* Compare Zeitschrift für Biologie, Band xix, p. 202. † Ibid., Band xx, p. 40.

‡ According to Hammarsten.

which had been dissolved in water after driving off the alcohol, became simply a little darker, but not rose-colored or violet. Hence heteroglobulose is to be considered as belonging to the anti group.

2. Protoglobulose, which still contained some heteroglobulose, when treated in the same manner with trypsin, behaved similarly but afforded besides an abundance of leucin, also some tyrosin and an extract which became deep violet on the addition of bromine water. Hence protoglobulose gives evidence of belonging to the hemi group.

Finally, we submitted to the digestive action of trypsin the third and fifth precipitates (so-called parapeptone) which were separated in continually decreasing quantities by neutralization, after renewed, energetic pepsin digestion of the original globulin. Both failed to yield any coagulum during their digestion with 0·3 per cent. sodium carbonate, and after the trypsin had acted for fourteen days, neutralization with acetic acid yielded a heavy precipitate, while considerable antipeptone was found in the solution. Although the digestion of the third neutralization precipitate still afforded a trace of leucin and tyrosin without giving any reaction with bromine, no leucin, tyrosin or a substance colored by bromine water could be obtained from the precipitate separated after the fifth pepsin digestion.

Hence globulin, like fibrin and other albuminous bodies, yields during pepsin digestion at the last only bodies of the anti group, which are peptonized, though slowly, by trypsin, but yield no further cleavage products.

Peptones. By W. Kühne and R. H. Chittenden.

Since there has been discovered in neutral ammonium sulphate a means for the complete precipitation of the albumose bodies, we have been induced to take up anew our former investigations on the behavior and composition of peptones. As these latter bodies are not precipitated by the ammonium salt, we had expected to obtain peptones free from the primary cleavage products of albumin and thereby advance another step in our knowledge of the definite products of the proteolytic action both of pepsin and of trypsin. Renewed investigation was demanded by the probability that hitherto pepsin-peptones entirely free from albumose have never been obtained, for such peptones as are to be found in commerce or in the hands of the most careful investigator of gastric digestion can readily be shown to contain albumose by saturating a solution of the preparation with ammonium sulphate. There will result an abundant precipitate of albumose and a surprisingly small residue of non-precipitated peptones or the entire absence of such a residue. Only antipeptone obtained by trypsin digestion will occasionally form an exception, and even then in most cases we cannot but doubt that the peptones so formed are wholly free from albumose.

In order to be certain of the presence of peptones in a digestive fluid, it must be made slightly acid with acetic acid, rubbed up with ammonium sulphate till saturated and then filtered from the excess of salt and the albumose precipitate. If the filtrate is thereupon treated with a large excess of strong sodium hydroxide and then a few drops of very dilute cupric sulphate be added, the appearance of the rosy red color of the biuret reaction will indicate the presence of peptones. If peptones are absent the fluid will be pure blue without a tinge of violet, since the solution can contain no other albuminous body. Even after an apparently energetic pepsin digestion the latter result is not at all rare, and a heavy precipitate by the ammonium salt is so frequently seen, that it is still to be doubted whether there is a pepsin-acid digestion which causes the disappearance of all albumose. On the contrary, the albumose precipitate after a sufficiently long and energetic trypsin digestion is very slight and peptone is to be found abundantly in the solution.

We have endeavored to prepare pure peptones in quantity from the solution saturated with ammonium sulphate. For this purpose the solution was first freed from the greater part of the salt by concentration and crystallization. During this process a small amount of a nitrogenous substance separated, perhaps albumose formed again from peptones when the solution was vigorously boiled and the temperature rose to 110° C. The mother liquor, after suitable dilution, was boiled with hot saturated baryta water until all ammonia was expelled, during which operation the precaution was taken to use no excess of barium hydroxide and thus decompose the peptones.

From time to time, therefore, portions were filtered, tested for sulphate, and when this became small in amount the last portions of sulphuric acid were removed by barium carbonate. From the filtrate, which always contained much barium, the latter was entirely removed by dilute sulphuric acid, either immediately or after a previous purification of the barium peptone compound. The peptones were then precipitated with alcohol and occasionally further purified with phosphotungstic acid. Naturally the large amount of ammonium sulphate to be removed formed a correspondingly troublesome quantity of barium sulphate, which could be handled only in large filtering bags, and occasioned a large loss of peptone in spite of a most careful washing of the precipitate with boiling water and the application of pressure. On evaporating the peptone solution, which contained but little salt, no resinous precipitate resembling albumose was to be seen.

1. *Amphopeptone.*

We have designated as amphopeptone the end product of the digestion of albumin by pepsin and acid. The first attempt to obtain this peptone free from albumose and in a quantity in some degree proportionate to our wants, showed us that there was needed not only the most active digestive fluid possible and long exposure to a temperature of 40° C. but also a very large amount of pepsin. Such a quantity of the ferment could be procured, however, only by first dissolving considerable quantities of the mucous membrane of the stomach in acid; quantities which must be taken into consideration, in addition to the fibrin to be digested, since something is formed in the self-digestion of the mucous membrane which necessarily remains mixed with the peptone. It is known that mistakes have already been committed by not distinguishing the products arising from the material of the mucous membrane, from those derived from the digested substance. For example, Hoppe-Seyler's erroneous asser-

tion that pepsin digestions yield leucin and tyrosin, rests wholly upon this circumstance, for since the digestion of the mucous membrane always commences with the disappearance of a mucilaginous substance, the derivatives of the latter must necessarily be expected in the resultant solution. Probably for this reason, artificial gastric juice which has been prepared from mucous membrane and is no longer mucilaginous, gives a precipitate when treated with alcohol which differs much from the precipitates of albumose and peptone in being almost as elastic as rubber and, as a rule, forming when shaken, a single ball in which the pepsin is then ordinarily inclosed. We have not yet examined this substance closely, since in the course of the investigations to be described, another more suitable method for precipitating and isolating the ferment has been discovered. We shall designate this elastic body for convenience, mucin-peptone. This mucin-peptone might possibly conceal the whole amount of peptone expected from the digested fibrin, or remain mixed with the latter in considerable quantity. In spite of this objection, which we at no time lost sight of, we prepared a quantity of fibrin-peptone without attempting to remove or to prevent the mixture in question. The observations made by Dr. Pollitzer* in the Physiological Institute at Heidelberg, on the influence of pepsin-peptone free from albumose on coagulation of the blood, were performed with such amphopeptone, which is not perfectly pure.

Supported by the following analyses of this peptone in our presumption that it was rendered impure by mucin-peptone, we sought a process that would exclude this impurity. This was found almost of itself after we had noticed that ammonium sulphate invariably precipitated from the acid solutions, in addition to albumose, the entire quantity of active pepsin. While, therefore, nothing capable of digestion with acids could in any way be obtained from the filtrates, an exceedingly active juice was formed by dissolving the precipitate in dilute hydrochloric acid. Hereafter, we accordingly prepared the strong pepsin solution, by simply precipitating large quantities of very concentrated gastric juice containing 0·5 per cent. hydrochloric acid with ammonium sulphate and dissolving the resinous precipitate, which did not contain an objectionable quantity of albumose bodies, in fresh dilute acid. By this means the mucin-peptone was gotten rid of, since it could not be precipitated by ammonium sulphate and thus a new method was found for preparing and isolating pepsin, which we shall enter upon at another time.

* Verhandl. d. Naturhist. med. Verein zu Heidelberg, N. F. III, p. 293.

1. *Amphopeptone prepared with ordinary gastric juice.*

Gastric juice, prepared from 145 grams of isolated mucous membrane from the fundus of pigs' stomachs by two days self-digestion in two litres of 0·4 per cent. hydrochloric acid, was added to 585 grams of well washed and boiled fibrin, previously swollen in four litres of acid of the same strength and the whole warmed for two days more at 40° C. The thin fluid-like mixture so obtained, was neutralized with sodium hydroxide and then filtered from the undissolved residue of the mucous membranes (nuclei of the gland cells) and the slight neutralization precipitate. After being made slightly acid with acetic acid, the fluid was heated to boiling, evaporated to two litres, then saturated with neutral ammonium sulphate, separated from the slight coagulum and precipitated albumose, again concentrated to one litre and freed from a large portion of the ammonium sulphate by crystallization at 0° C. In order to still further separate the salt, the solution was treated with one litre of absolute alcohol, again placed in the cold, and finally strained through linen to remove the fine powdery salt, which was wholly free from precipitated peptone. After having been freed from alcohol, by vigorous boiling and concentration to the consistency of syrup and from much salt by crystallization, the thick fluid was filtered by suction, boiled after much dilution with a large amount of barium carbonate until the odor of ammonia had vanished. From the solution, separated from the barium sulphate and again much concentrated, alcohol precipitated the peptone as a barium compound which could be freed from salts (especially sodium chloride) by repeated precipitation and boiling with alcohol. Finally the barium-peptone was decomposed as much as possible with dilute sulphuric acid. As was seen later from the concentrated peptone solution, there remained dissolved a trace of sulphuric acid, but only enough to make the fluid assume a slight opalescence after boiling with barium chloride and hydrochloric acid. An attempt was made to purify the isolated peptone by evaporating, precipitating with alcohol, dissolving in water and reprecipitating with alcohol. This did not succeed well, as shown later by the high percentage of ash. By drying first on a water bath, then in an air bath at 105° C. with frequent stirring, which destroyed the firm resinous surface, the peptone gradually became solid, and changed to a puffed up mass. The resulting product could be ground, when cold, to a light, very hygroscopic powder and weighed in this condition 25 grams.

In an attempt to dry the substance for analysis, during which the temperature was allowed to rise to 110° C. it was found impossible to obtain a constant weight, perhaps on account of decomposition setting in, as suggested by an unpleasant odor which had begun to develop while on the water bath. Portions of 8–10 grams lost daily 0·03–0·04 gram. The analyses were accordingly made only after drying many days. Portions purified with alcohol, dissolved in boiling water with addition of hydrochloric acid, gave no reaction to be distinguished when heated with barium chloride.

Carbon, hydrogen and nitrogen were determined as before, the sulphur by a method already used by us to some extent,* viz: by fusion with potassium hydroxide and potassium nitrate according to the method distinguished by Hammarsten as 1*a*.†

The results of the analysis (Amphopeptone A), shown by the following table, were hardly satisfactory and the low percentage of carbon, particularly, was quite a surprise to us, hence we proceeded at once to the previously mentioned preparation of a peptone, which would probably be rendered less impure by derivatives of the mucous membrane and which would, moreover, be easier to purify further.

2. *Amphopeptone prepared with purified pepsin.*

Preparation of the pepsin.—1220 grams of isolated mucous membrane from the fundus of ten pigs' stomachs were warmed at 40° C. with seven litres of 0·5 per cent. hydrochloric acid for six days. The mixture was then saturated directly with ammonium sulphate, by which a resinous precipitate, with large, sticky lumps was formed, easily collected on a cloth filter. After pressing out the salt solution as much as possible and washing with water, the gummy mass was dissolved in five litres of 0·4 per cent. hydrochloric acid and warmed again at 40° C. for a few days. Then for the first time it was filtered through paper. As preliminary experiments had shown that gastric juice which contains small quantities of ammonium sulphate molds easily, the second digestion and the following fibrin digestion were carried on in the presence of 0·25 per cent. of thymol, which wholly prevented the formation of mold. The mass submitted for the second time to self-digestion, gave now with the ammonium salt a much smaller precipitate, which contained only a very

* Compare our earlier papers. Zeitschrift für Biologie, vols. xix and xx.

† Zeitschrift für physiol. Chem., vol. ix, p. 288.

AMPHOPEPTONE (A).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash %	$BaSO_4$ from the ash. gram.	S from ash %
I	0·5615	0·3020	6·05	0·8406	40·82	----	--	----	----	----	----	-----	----	----	-----	----
II	0·4269	0·2267	5·90	0·6420	41·01	----	--	----	----	----	----	-----	----	----	-----	----
III	0·5601	0·3005	5·96	0·8410	40·95	----	--	----	----	-----	----	-----	----	----	-----	----
IV	0·5622	-----	----	-----	----	70·0	4·2	757·1	15·84	-----	----	-----	----	----	-----	----
V	0·4042	-----	----	-----	----	50·2	4·6	764·2	15·42	-----	----	-----	----	----	-----	----
VI	0·5174	-----	----	-----	----	----	--	----	----	0·0426	8·23	-----	----	----	-----	----
VII	0·5625	-----	----	-----	----	----	--	----	----	0·0450	8·00	-----	----	----	-----	----
VIII	0·4930	-----	----	-----	----	----	--	----	----	-----	----	0·0295	0·82	0·59	-----	----
IX	9·7954	-----	----	-----	----	----	--	----	----	-----	----	0·0550	0·94	0.71	-----	----
X	1·0799	-----	----	-----	----	----	--	----	----	-----	----	-----	----	----	0·0183	0·23

Percentage composition of the ash-free substance.

								Average.
C	44·42	44·62	44·55	----	----	----	----	*44·53*
H	6·58	6·42	6·48	----	----	----	----	*6·49*
N	----	----	----	16·69	16·78	----	----	*16·73*
S	----	----	----	----	----	0·66	0·78	*0·72*
O	----	----	----	----	----	----	----	*31·53*
								100·00

small amount of albumose bodies, while a portion digested as a test for the third time, gave in the filtrate from the precipitate produced by the ammonium salt, so faint a biuret reaction for peptones that it was plainly evident, that the slight residue of albumins from the mucous membrane now remaining, could be overlooked without danger.

Digestion of the fibrin.—3800 grams of washed but not boiled fibrin were digested with the twice precipitated pepsin, which was dissolved in ten litres of 0·4 per cent. hydrochloric acid. To obtain as little albumose and as much peptone as possible, the mixture was allowed to remain at 37°–40° C. for two weeks. At the end of that time, filtered portions gave only slight precipitations by neutralization, but a heavy precipitate was obtained with ammonium sulphate, with sodium chloride, with sodium chloride and acetic acid, and still further by sodium chloride and nitric acid or metaphosphoric acid. Nevertheless the filtrate saturated with ammonium sulphate contained much peptone.*

Preparation and purification of the peptone.—For this purpose the filtrate was neutralized with sodium hydroxide, filtered through linen, especially for removing the impurities of the fibrin, the filtrate slightly acidified with acetic acid, concentrated to about four litres, precipitated with an excess of ammonium sulphate, filtered and pressed, the solution boiled with barium hydroxide and finally with barium carbonate and a large quantity of water, until ammonia could no longer be detected. The barium sulphate was then removed by filtration through cloth bags which were repeatedly washed and pressed, the solution evaporated to about four litres, the barium-peptone decomposed with a very slight excess of sulphuric acid, the new precipitate of barium sulphate filtered off, the solution concentrated to two litres, the free acid neutralized with ammonia and after cooling, six per cent. English sulphuric acid (previously diluted) was added; then the sulphuric acid-peptone solution was precipitated with a large excess of phosphotungstic acid, the precipitate washed first with six per cent. sulphuric acid, then with a large quantity of water, after which the compound was decomposed with excess of barium hydroxide and the excess completely removed from the fil-

* Later experiments have shown that pepsin acts much more energetically if the ammonium sulphate is completely removed by dialysis, before each new solution and digestion of the pepsin-containing precipitate in hydrochloric acid, and further, that *nearly pure pepsin* becomes wholly inactive by being warmed with dilute hydrochloric acid in the presence of even small quantities of ammonium sulphate.

trate with sulphuric acid. The peptone solution thus obtained had a distinctly acid reaction, and strange to say, contained hydrochloric acid, which was hardly to be expected after the very careful washing which the precipitate had received. The solution was neutralized with ammonia to render the acid harmless on concentration. Then we succeeded in obtaining the evaporated residue free from ammonium chloride by repeated precipitation and boiling with alcohol.

As already mentioned, the method gives rise to much loss and the same holds true of the otherwise excellent precipitation of peptone by phosphotungstic acid according to the method of Hofmeister, for so far as our experience extends, peptones cannot be *completely* precipitated in this manner. There arises in the filtrate, containing excess of phosphotungstic acid, not only additional turbidity and precipitation due to peptone, but considerable quantities of peptone are still found in the liquid, which has perhaps remained clear for months, if treated with barium hydroxide;—a circumstance which we were not able to prevent even by strongly acidifying the solution to be precipitated with phosphotungstic acid, with either sulphuric or hydrochloric acid.

Behavior of the peptone.—This peptone was also difficult to convert into a dry state, although we did succeed ultimately in bringing it to a constant weight as a fine, exceedingly hygroscopic powder, by heating for some time at 105° C. *in vacuo.* The first difficulty was found in commencing the drying, for although we treated the glue-like mass repeatedly with absolute alcohol, then for a long time with ether and finally boiled it again with alcohol, thereby changing it into an almost dry, crumbling condition, we were compelled at last to stop its further direct drying, since at 100° C. the preparation took on the consistency of pitch and formed a bulky foam from which alcohol vapor continually escaped. Therefore the alcohol was first driven out by thorough boiling with water and the latter removed as much as possible at 100° C. This was also a tedious performance for, although the substance no longer foamed up so violently, it did not become dry until after many days of stirring and breaking the covering which continually bubbled up. The same proceeding was repeated, although in a less degree, on transferring the substance to the air bath at 105° C., and only the single portions taken for analysis could be brought to a constant weight without puffing up further at 105° C.

While drying, the unpleasant odor noticed from amphopeptone A was also observed here, although only in a slight degree.

The peptone thus obtained appears (when dried at 105° C.) as a dry, light yellowish powder. It can be preserved in this form only when most tightly stoppered. In the air it soon forms large balls, becomes sticky like pitch and melts to a tough mass which does not become visibly thinner. What is truly surprising is the behavior of the peptone towards water. *A bit of the powder wet with a small drop of water hisses and steams like phosphoric anhydride when moistened, and when this, or the powdered but not absolutely dry preparation which no longer hisses, is dissolved in water, a development of heat is to be noticed.* We have observed the same remarkable peculiarity in anti-peptone to be described later.

Analysis of the preparation (Amphopeptone B) dried at 105° C. *in vacuo*, gave the results tabulated in the accompanying table.

Amphopeptone (b).

After these results were obtained, an attempt was made to reduce the ash content of the preparation by repeated precipitation with alcohol, which succeeded so well that the substance when dried *in vacuo* over sulphuric acid, later at 106° C. until of constant weight, contained then 2·15 per cent. of ash instead of 3·25 per cent.

I. 0·5500 gram of this preparation gave 0·3400 gram H_2O = 6·86 per cent. H and 0·9566 gram CO_2 = 47·43 per cent. C.

II. 0·7121 gram of substance gave 98·4 c. c. N at 16·4° C. and 765·5mm. pressure = 16·49 per cent. N.

III. 0·7839 gram substance gave 0·0169 gram ash = 2·15 per cent. of ash.

Therefore in the ash-free substance (Amphopeptone b) there are—

48·47% C, 7.02% H, 16·86% N.

II. *Antipeptone.*

As with amphopeptone, we have formed in various digestion experiments several preparations of antipeptone by the action of trypsin. These preparations have been studied both after purification with alcohol and after further purification with phosphotungstic acid, but in every case after complete removal of the albumose bodies. We have not attempted to meet the objection that the antipeptone is not formed exclusively from the digested fibrin, but in part from the albumins of the pancreas. This would have necessitated experiments with pure trypsin, which seemed too costly to undertake. Instead of this we have studied a peptone which we shall distinguish

AMPHOPEPTONE (B).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash %	$BaSO_4$ from the ash. gram.	S from the ash %
I	0·8347	0·5320	7·08	1·4379	46·97	----	---	----	----	-----	----	-----	----	----	-----	----
II	0·3511	0·2176	6·88	0·6097	47·35	----	---	----	----	-----	----	-----	----	----	-----	----
III	0·4170	-----	----	-----	47·21	----	---	----	----	-----	----	-----	----	----	-----	----
IV	0·4694	-----	----	-----	----	63·56	21.2	758·2	15·74	-----	----	-----	----	----	-----	----
V	0·6948	-----	----	-----	----	93·88	21·0	758·8	15·75	-----	----	-----	----	----	-----	----
VI	0·6452	-----	----	-----	----	----	---	----	----	0·0210	3·25	-----	----	----	-----	----
VII	0·6712	-----	----	-----	----	----	---	----	----	0·0215	3·20	-----	----	----	-----	----
VIII	0·5032	-----	----	-----	----	----	---	----	----	-----	----	0·0360	0·98	0·68	-----	----
IX	0·5936	-----	----	-----	----	----	---	----	----	-----	----	0·0477	1·10	0·80	-----	----
X	1·3164	-----	----	-----	----	----	---	----	----	-----	----	-----	----	----	0·0288	0·30

Percentage composition of the ash-free substance.

								Average.
C	48·54	49·93	48·79	----	----	----	----	*48·75*
H	7·31	7·11	----	----	----	----	----	*7·21*
N	----	----	----	16·26	16·27	----	----	*16·26*
S	----	----	----	----	----	0·71	0·83	*0·77*
O	----	----	----	----	----	----	----	*27·01*
								100·00

as *gland peptone*, being derived exclusively from the self-digestion of the albuminous bodies of the gland substance, without any addition of fibrin or other albumin.

How superior the action of trypsin is to that of pepsin, is seen not alone in the total decomposition of hemipeptone which is accomplished only by the former, but also in the incomparably more rapid and perfect change of albumose to peptone. Hence the particularly troublesome and tedious treatment of antipeptone with ammonium sulphate may well seem superfluous when there is no albumose present. We must however be perfectly sure, by a preliminary test with that salt, that albumose is absent and its use is unavoidable where impure trypsin is employed in large quantities; that is, where an infusion of the pancreatic gland or the so-called artificial pancreatic juice is used. From the latter, ammonium sulphate precipitates a mixture which contains besides unaltered, highly active trypsin, whose isolation we propose later to study in this way, various other bodies, such as albumose, whose removal is necessary in the preparation of pure peptone.

Antipeptone (C).

Preparation of the pancreatic juice.—100 grams of dried ox pancreas, purified with alcohol and ether, were warmed at 40° C. with 500 c. c. of 0·1 per cent. salicylic acid for 12 hours, and filtered through muslin. The residue was then mixed with 500 c. c. of 0·25 per cent. sodium carbonate, a little thymol added and the mixture again warmed at 40° C. for 12 hours. The acid solution, after it had been neutralized, was brought to the same degree of alkalinity with sodium carbonate, a little thymol added and also warmed at 40° C. for the same length of time. After filtering and pressing the residue of tissue, both filtrates were united. The weight of the undissolved residue, dried at 100° C., amounted as usual to 12 grams. Thus the nuclei of the cells, the collagen and the portion of elastin undigested under these conditions, is equal to 12 per cent. of the dry pancreas, freed from fat.

Digestion of the fibrin.—300 grams of dry fibrin, purified by washing and boiling with water, then with alcohol and finally by extraction with ether were softened with boiling water (when the weight amounted to 970 grams after squeezing with the hands), then warmed at 40° C. with 3 litres of 0·25 per cent. sodium carbonate containing 0·5 per cent. of thymol. To this was added the whole

infusion obtained from the 88 grams of self-digested pancreas, after which the mixture was continued at 40° C. for six days. At the end of the first day nearly all of the fibrin had disappeared, although a considerable portion appeared to float on the surface of the fluid. When examined more closely, however, this residue proved to be extremely light, hollow, easily crushed and with a somewhat greasy feeling. A similar residue, the amount of which we did not determine, remained at the end of six days and consisted mainly of antialbumid with much tyrosin.

Preparation of the antipeptone.—The solution resulting from the above digestion was made slightly acid with acetic acid, boiled, passed through a filtering bag, concentrated to 1 litre, and freed from a large amount of leucin and tyrosin by crystallization and filtration. The resultant, brownish-looking syrup was treated with alcohol until peptones began to precipitate, and after the latter had been redissolved by boiling the solution, it was placed aside for crystallization. The filtrate, which now contained only a small amount of amido acids, was freed from alcohol by boiling, diluted with a saturated solution of ammonium sulphate, which had also served for washing out the mass of crystals on the filters, and then completely saturated with the ammonium salt in substance. After separating the slight precipitate so formed, in which some leucin and tyrosin was detected, the greater portion of the ammonium salt was removed from the filtrate by repeated concentration and crystallization, while the remainder was gotten rid of, as before, with barium hydroxide and barium carbonate. Since in this case, precipitation with phosphotungstic acid could not yet be employed, we attempted to purify the peptone as much as possible from other products of digestion (amido acids), first, as a barium compound by repeated precipitation and boiling with alcohol, after which the barium-peptone was exactly decomposed with sulphuric acid and the free peptone purified in a similar manner by repeated precipitation and extraction with alcohol, once or twice in the presence of a little acetic acid. The peptone thus obtained, when dried at 105° C., weighed 120 grams. Assuming that albuminous bodies by complete typsin digestion, split up into 50 per cent. of products arising from the wholly decomposable hemipeptone and 50 per cent. of antipeptone not further changed by typsin, then the amount obtained—120 grams—agrees with this so far as it is possible, with the unavoidable losses which the treatment of large quantities in this manner implies. The 388 grams of dry albumin (300 grams of fibrin and 88 grams of self-

digested material from the pancreas) would have had to yield 194 grams of antipeptone if there were no loss. The loss, however, of 74 grams noticed in our experiment is sufficiently explained by the noticeable solubility of peptone in the water contained in alcohol, and by the conversion of a portion of the peptone into antialbumid.

Behavior of the antipeptone.—This peptone was still more difficult to dry than the amphopeptone formed by pepsin digestion, and it could only be accomplished after the removal of all alcohol by thorough boiling with water. As the solution became very concentrated on the water-bath, hydrogen sulphide, as shown by reaction with lead acetate, was given off together with a strong odor of valerianic acid, which was also evolved quite noticeably at 105° C. In order to obtain a constant weight it was necessary to dry the mass at 110° C.

The analysis of the product is shown in the accompanying table.

Antipeptone (D).

The behavior of the preceding preparation while being dried, naturally suggested the suspicion that the substance was either decomposable at 100° C. or less in the air, or else that it contained some decomposable admixture. We therefore attempted a further purification of antipeptone and at the same time a more cautious method of drying.

For this purpose another preparation of antipeptone was made in the following manner.

230 grams of commercial dry pancreas, somewhat less active than that employed in the preceding preparation, were warmed at 40° C. for three hours with 1200 c. c. of 0·1 per cent. salicylic acid, after which the mixture was neutralized with sodium carbonate and to it was added directly 1920 grams of boiled, moist fibrin, 82 grams of dry sodium carbonate and 32 grams of thymol. This mixture was warmed at 40° C. for seven days, at the end of which time the residue of the pancreas, the antialbumid produced, and considerable separated tyrosin, formed a noticeable sediment, which was filtered off and pressed, after the residue had been thoroughly washed with water warmed at 40° C. The filtrate was made slightly acid, heated to boiling, and as this produced only a slight precipitate it was immediately concentrated to about three litres. On cooling, an abundant slate-colored precipitate separated, composed almost entirely of tyrosin. After removing this by filtration, the solution was saturated with ammonium sulphate and the resultant filtrate treated as in the

ANTIPEPTONE (C).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash %
I	0·5784	0·3323	6·38	0·9503	44·80	....	....	....	....	....	....	....	....	....	....	....
II	0·5949	0·3412	6·73	0·9776	44·81	....	....	....	....	....	....	....	....	....	....	....
III	0·5771	....	....	....	....	74·36	5·0	763·0	15·95	....	....	....	....	....	....	....
IV	0·4463	....	....	....	....	57·45	4·8	762·0	15·93	....	....	....	....	....	....	....
V	0·5256	....	....	....	....	....	....	....	....	0·0278	5·28	....	....	....	....	....
VI	0·5092	....	....	....	....	....	....	....	....	0·0270	5·32	....	....	....	....	....
VII	1·0348	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0·17
VIII	0·5998	....	....	....	....	....	....	....	....	....	....	0·0368	0·84	0·67	0·0130	....
IX	0·6149	....	....	....	....	....	....	....	....	....	....	0·0390	0·87	0·70	....	....

Percentage composition of the ash-free substance.

							Average.
C	47·30	47·31	....	....	....	....	*47·30*
H	6·74	6·72	....	....	....	....	*6·73*
N	....	....	16·84	16·82	....	....	*16·83*
S	....	....	....	....	0·71	0·74	*0·73*
O	....	....	....	....	....	....	*28·41*
							100·00

previous case; that is, a barium peptone compound was formed, purified with alcohol and this exactly decomposed with sulphuric acid.

In order to separate the free peptone, the solution was concentrated at a gentle heat with the addition of a little ammonia, precipitated and boiled with alcohol, the almost liquid precipitate dissolved in water, the solution acidified with acetic acid, concentrated again, precipitated and extracted hot with alcohol, repeatedly washed and kneaded with ether, kept for a long time under ether and then slowly dissolved in as small an amount of cold water as possible. When filtered, a small residue of tyrosin appeared in the preparation. The new solution was concentrated at a gentle heat, precipitated again with alcohol, the peptone boiled and washed with alcohol, allowed to stand for some time with a large quantity of absolute alcohol, again treated with ether as before, and as it had now become friable, it was immediately dried in vacuo over sulphuric acid. On attempting to dry it at 100° C. the melting mass foamed so much that it had to be dried in the air. When this had been done with frequent stirring on the water bath for several days, the mass was finally dried completely *in vacuo*, first at 100° C., then at 105° C. A few weeks of this drying were needed to bring the substance to a constant weight, and in order to prepare the various quantities for analysis they had to remain *in vacuo* over sulphuric acid and at 105° C. for some time. Probably in consequence of the thorough treatment with ether, the preparation when warmed gave much less odor than the former one.

Composition of Antipeptone (D).

I. 0·5061 gram substance gave 0·2875 gram H_2O = 6·31 per cent. H and 0·7937 gram CO_2 = 42·76 per cent. C.

II. 0·4449 gram substance gave 0·2527 gram H_2O = 6·31 per cent. H and 0·7024 gram CO_2 = 43·05 per cent. C.

III. 0·5010 gram substance gave 0·2870 gram H_2O = 6·36 per cent. H and 0·7885 gram CO_2 = 42·91 per cent. C.

IV. 0·4414 gram substance gave 56·0 c. c. N at 21·0° C. and 759·2 mm. pressure = 14·91 per cent. N.

V. 0·5870 gram substance gave 76·5 c. c. N at 21·6° C. and 758·0 mm. pressure = 15·13 per cent. N.

VI. 0·6930 gram substance gave 0·0694 gram ash = 10·01 per cent.

VII. 0·5017 gram substance gave 0·0504 gram ash = 10·04 per cent.

VIII. The ash from 0·5017 gram substance gave 0·0325 gram $BaSO_4$ = 0·89 per cent. S calculated on the original substance.

On account of the large percentage of ash and the large amount of sulphates, the sulphur of the organic matter was not determined.

Percentage composition of the ash-free substance.

						Average.
C	47·52	47·83	47·69	----	----	47·68
H	7·01	7·01	7·07	----	----	7·03
N	----	----	----	16·57	16·80	16·68

Antipeptone (E).

In order to obtain a still purer preparation and especially to free it from the large percentage of ash, a portion of antipeptone (D) was dissolved in boiling water after the first treatment with ether, then when cold the solution was acidified with sulphuric acid to such an extent that it contained 6 per cent. of acid and precipitated with a large excess of phosphotungstic acid. The precipitate, after the manner already described under amphopeptone (B), was washed thoroughly with dilute sulphuric acid and then with water, finally decomposed with baryta, the barium-peptone precipitated with alcohol, washed, the alcohol driven off by heat, the aqueous solution of the compound exactly decomposed with sulphuric acid, the solution concentrated after the addition of a few drops of ammonia, precipitated with alcohol, dissolved again in water, concentrated with the addition of a little acetic acid, again precipitated with alcohol, and the product so obtained treated thoroughly with alcohol and ether in the same manner as preparation D, and finally dried in the same manner as that. The substance so prepared, was lighter colored than the preceding, not quite so hygroscopic, and in drying gave scarcely any odor.

The analysis of the product is shown in the accompanying table.

Antipeptone (F). (*Gland peptone.*)

This peptone was obtained as a bye product in a preparation of trypsin from 1,000 grams of dry pancreas and was formed wholly from the albuminous bodies of the gland substance, after extraction with alcohol and ether. It is not probable that the peptone contained, in any considerable quantity, any products from the digestion of elastin, since the elastic tissue could have been but little altered under the conditions in which the self digestion of the gland took place during the preparation of the infusion, and furthermore there would have been needed for solution in the latter, a finer subdivision

ANTIPEPTONE (E).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash %
I	0·6561	0·3822	6·47	1·0840	45·05	....	....	....	....	....	....	....	....	....	....	....
II	0·6892	0·3989	6·43	1·1300	44·71	....	....	....	....	....	....	....	....	....	....	....
III	0·3911	....	....	....	....	59·68	22·8	756·7	17·61	....	....	....	....	....	....	....
IV	0·7900	....	....	....	....	120·0	21·4	757·7	17·63	....	....	....	....	....	....	....
V	0·6379	....	....	....	....	96·63	21·4	757·6	17·58	....	....	....	....	....	....	....
VI	0·5796	....	....	....	....	....	....	....	....	0·0213	3·67	....	....	....	....	....
VII	0·6332	....	....	....	....	....	....	....	....	0·0233	3·68	....	....	....	....	....
VIII	0·9190	....	....	....	....	....	....	....	....	....	....	0·0500	0·74	0·63	....	....
IX	0·5438	....	....	....	....	....	....	....	....	....	....	0·0300	0·76	0·65	....	....
X	1·2128	....	....	....	....	....	....	....	....	....	....	....	....	....	0·0100	0·11

Percentage composition of the ash-free substance.

								Average.
C	46·77	46·42	....	....	....	....	....	*46·59*
H	6·71	6·67	....	....	....	....	....	*6·69*
N	....	....	18·28	18·31	18·25	....	....	*18·28*
S	....	....	....	....	....	0·66	0·68	*0·67*
O	....	....	....	....	....	....	....	*27·77*
								100·00

and long continued action. However this may be, the albumins of the pancreas naturally cannot be classified with the substances ordinarily used in digestion experiments, such for example as fibrin, without further investigation, for although there may be substances in the gland cells like serum-albumin, globulin and myosin, there are also many bodies quite different from these, as, for example, the leucoid precipitated by excess of acetic acid, zymogen and trypsinogen, all of which are decomposed by self-digestion and yield amido acids and peptones. So long as trypsin digestions are not ordinarily conducted with pure trypsin, it is of especial interest to find out the composition of the gland peptones, which, as a rule, have invariably been mixed in greater or less quantity with the antipeptones hitherto investigated.

Preparation.—1,000 grams of dry pancreas were warmed at 40° C. for twelve hours with five litres of 0·1 per cent. salicylic acid and 0·25 per cent. of thymol, filtered through muslin, the residue warmed another twelve hours with two litres of 0·25 per cent. sodium carbonate and 0·5 per cent. of thymol, again filtered and pressed, the two fluids united, brought up to an alkalinity of 0·25 per cent. of sodium carbonate and then warmed at 40° C. for three days.

After filtering through paper, the whole solution was slightly acidified with acetic acid and then saturated with five kilos. of ammonium sulphate, by which means there was precipitated a little albumose and all of the trypsin, the further treatment of which is of no interest here, while the gland peptone remained in solution. It is to be noticed in the separation of this peptone that it was treated exactly like preparation (C), excepting that the second purification with ether could be omitted. The preparation, after drying for some time, left a small residue of tyrosin when dissolved in cold water. It was therefore precipitated from this solution with alcohol, then freed from alcohol by boiling with water, dried directly over a water-bath and finally *in vacuo* at 106° C. until a constant weight was obtained.

The analysis of the product is seen in the following table.

Antipeptone (G). (Gland peptone.)

This preparation was obtained from the preceding product by the following process; the solution of the peptone was acidified with 6 per cent. of sulphuric acid, precipitated with a large excess of phosphotungstic acid, the precipitate carefully washed, then decomposed with barium hydroxide, the latter exactly removed with dilute sul-

ANTIPEPTONE (F) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash. %
I	0·7511	0·4632	6·79	1·1652	41·97	....	..	....	....	....	....	....	....	....	....	....
II	0·3783	0·2301	6·75	0·5830	42·02	....	..	....	....	....	....	....	....	....	....	....
III	0·5729	....	....	....	....	79·12	18·4	752·0	16·08	....	....	....	....	....	....	....
IV	0·4792	....	....	....	....	66·36	18·6	754·0	16·15	....	....	....	....	....	....	....
V	0·6459	....	....	....	....	....	..	....	....	0·0352	5·44	....	....	.. .	....	....
VI	0·6494	....	....	....	....	....	..	....	....	0·0367	5·65	....	....	....	....	....
VII	1·2953	....	....	....	....	....	..	....	....	....	....	....	....	....	0·0128	0·13
VIII	0·6509	....	....	....	....	....	.	....	....	....	....	0·0289	0·60	0·47	....	....
IX	0·6269	....	....	....	....	....	..	....	....	....	....	0·0270	0·59	0·46	....	....

Percentage composition of the ash-free substance.

							Average.
C	44·42	44·48	....	....	....	....	*44·45*
H	7·19	7·15	....	....	....	....	*7·17*
N	....	....	17·02	17·09	....	....	*17·06*
S	....	....	....	....	0·51	0·50	*0·50*
O	....	....	....	....	....	....	*30·82*
							100·00

phuric acid, the solution concentrated, the peptone precipitated and washed with alcohol and finally boiled with alcohol. The partially dry product was then dissolved in cold water, leaving a small amorphous residue which gave no reaction for tyrosin. The solution was then concentrated on the water-bath and again precipitated with alcohol, after which it was dried finally, over sulphuric acid in vacuo and at 106° C. in vacuo, until of constant weight.

The following table shows the results of the analysis.

Antipeptone (H). (Gland peptone.)

This product was prepared and purified in exactly the same manner as the former one (G), but was made from another trypsin preparation, in which a smaller amount of dry pancreas was used. When dissolved for the last time in cold water, some little insoluble matter remained (from which it was freed) which, however gave no reaction for tyrosin.

The following table shows the results of the analysis.

GENERAL PROPERTIES OF THE PEPTONES.

We should have liked to study more accurately the physical behavior of the different samples of peptones, especially their optical properties as determined by specific rotary power. It was easy to show that they were all laevo-rotary, but we have not yet succeeded in making any quantitative determinations of sufficient accuracy to be of value. The decidedly brown color of the solutions prevented the use of a sufficiently long tube, or a solution of the proper concentration, necessary to determine specific rotation.

It would be of still greater importance to investigate the rate of diffusion of peptones, it being more necessary from the fact, that previous observations on the diffusion of the products of digestion can have but little reference to pure peptones, but rather to the albumose bodies so long overlooked. We have, however, not as yet begun these investigations owing to lack of material. All of the peptones obtained by us in the dry state, showed considerable rise of temperature when moistened with, or dissolved in water.

It is worthy of notice, physiologically, that according to observations made in the physiological Institute at Heidelberg, by Dr. Pollitzer of New York, no one of the peptones would hasten or retard coagulation of the blood, either when injected into the veins or added to the shed blood, such action being due wholly to certain of the albumose bodies.

ANTIPEPTONE (G) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash. %
I	0·4914	0·3163	7·15	0·7600	42·17	....	..	....	....	....	....	....	....	....	....	....
II	0·6475	0·4141	7·10	0·9998	42·10	....	..	....	....	....	....	....	....	....	....	....
III	0·6381	....	....	....	....	94·8	17·6	762·2	17·58	....	....	....	....	....	....	....
IV	0·4756	....	....	....	....	69·6	17·4	763·8	17·37	....	....	....	....	....	....	....
V	0·6898	....	....	....	....	....	..	....	....	0·0132	1·91	....	....	....	....	....
VI	0·6732	....	....	....	....	....	..	....	....	0·0132	1·96	....	....	....	....	....
VII	1·3630	....	....	....	....	....	..	....	....	....	....	....	....	....	0·0087	0·09
VIII	0·8856	....	....	....	....	....	..	....	....	....	....	0·0249	0·38	0·29	....	....
IX	0·6059	....	....	....	....	....	..	....	....	....	....	0·0178	0·40	0·31	....	....

Percentage composition of the ash-free substance.

							Average.
C	42·99	42·93	....	....	....	....	*42·96*
H	7·29	7·24	....	....	....	....	*7·26*
N	....	....	17·70	17·91	....	....	*17·80*
S	....	....	....	....	0·30	0·32	*0·31*
O	....	....	....	....	....	....	*31·67*
							100·00

ANTIPEPTONE (H) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash. %
I	0·4107	0·2607	7·05	0·6548	43·47	----	----	----	----	----	----	----	----	----	----	----
II	0·5174	0·3243	6·96	0·8280	43·64	----	----	----	----	----	----	----	----	----	----	----
III	0·5940	----	----	----	----	88·48	18·6	762·8	17·58	----	----	----	----	----	----	----
IV	0·5117	----	----	----	----	----	----	----	----	0·0106	2·07	----	----	----	----	----
IVa	0·5117	----	----	----	----	----	----	----	----	----	----	----	----	----	0·0088	0·10
V	0·4969	----	----	----	----	----	----	----	----	----	----	0·0240	0·66	0·56	----	----

Percentage composition of the ash-free substance.

					Average.
C	44·39	44·56	----	----	*44·47*
H	7·20	7·11	----	----	*7·15*
N	----	----	17·94	----	*17·94*
S	----	----	----	0·57	*0·57*
O	----	----	----	----	*29·87*
					100·00

A few observations on the taste of peptones are of interest. While the genuine albumins and the albumose bodies excite practically no sense of taste, the less so the purer they are, it appears as if peptones belong to the most offensively tasting bodies. In order to see what an important change the taste of an albuminous body undergoes on digestion, and at the same time what the taste of peptones is, warm 50 c. c. of fresh milk to 40° C. and then add to it a small fragment of soluble trypsin (prepared from ox pancreas), which excites no taste of itself. The milk at first coagulates, then regains its former appearance by solution of the coagulum, but tastes no better than gall. Nevertheless we believe that the especially objectionable taste proceeds not from peptones, but from certain compounds heretofore only accidentally separated from them. For, among our preparations, which as a whole tasted something like roast meat, as if burnt, but above all nauseatingly bitter and astringent even in a 2 per cent. solution, we found one that in a 10 per cent. solution was free from this disagreeable peculiarity and had a pleasant, sweet taste like meat. It is to be noticed that this was the preparation of antipeptone (F) which had not been purified by phosphotungstic acid. Only by taking a large swallow was there noticed a not unpleasant taste, peculiar to certain cheeses after becoming alkaline.

The most important property for the separation and treatment of peptones is for the present, the lack of precipitation by a great number of reagents, which precipitate more or less perfectly albumins and albumose bodies, especially sodium chloride, either alone, or with the addition of acid. We have repeatedly confirmed the observations made by Wenz that even sodium chloride and acetic acid, sodium chloride and nitric acid or metaphosphoric acid do not completely precipitate the albumose bodies. In whatever proportion these additions may be made, there always remains at the end a solution which gives with alcohol a precipitate of salt, in which albumose is still to be found, or from which albumose may be separated by dialysing and concentrating, according to the method already used. The only perfect precipitant of these substances is ammonium sulphate. It is however an error to attribute to this salt the same action on peptones. Wherever peptones occur, they will always be found in the filtrate from a solution saturated with ammonium sulphate, and we must conclude from some opposed statements that in the experiments on which they are based, albumose bodies instead of peptones were present, since we are certain that by means of our new method, it can be generally shown what an unexpected difference exists between the ap-

parently vigorous action of a poorly prepared gastric juice, or commercial preparation of pepsin and the action of solutions actually rich in pepsin. Even where the fibrin almost instantly disappears, the amount of ferment may still be quite insufficient to produce noticeable traces of peptones. Therefore care must be taken not to conclude immediately from a speedy solution, that digestion has been complete, since this is to be determined only by the disappearance of the primary cleavage products of digestion, that is, the change of albumose into peptones.

It was especially interesting to ascertain whether peptones isolated according to the methods already described, were likewise precipitated by ammonium sulphate or by other reagents that precipitate the albumose bodies, a question which was interesting considering the oft-asserted formation of albumin or albumose from peptones. To our surprise we noticed in the beginning that both antipeptone and amphopeptone after complete purification, under certain circumstances gave rise to a turbidity or even a resinous precipitate, not only with ammonium sulphate, but also when their solutions were saturated with salt or when treated with acetic acid, nitric acid or metaphosphoric acid, just as if albumose had been formed or the albumose not completely removed by the previous treatment. Even if these precipitates concerned only a small part of the material in solution, their appearance would need explanation. So far as we can now determine, the occasion of this behavior is a circumstance concerning which we do not care to decide whether it really depends on the formation of albumose from peptone or not. It is to be observed that if the purification of the peptone by sulphuric acid is conducted incautiously, either on decomposing the barium-peptone or on acidifying before precipitation with phosphotungstic acid, the appearance afterward of albumose is avoided provided the solutions, when warm, are never exposed to an excess of acid. That a resinous precipitate appears, while boiling the solution saturated with ammonium sulphate at 110° C. has already been mentioned, but this cannot cause any impurity of the peptone remaining in solution, any more than the well known precipitation of antialbumid during trypsin digestion can occasion an impurity of the antipeptone.

Since the fact is proved that peptones are not precipitated by ammonium sulphate, these bodies are then characterized more than ever by the property long attributed to them of being rendered turbid by very few reagents and completely precipitated by a still more limited number. A list of the latter reagents includes only tannin

and mercuric iodide in potassium iodide, while imperfect precipitants of peptones are phosphotungstic acid or phosphomolybdic acid and picric acid.

The following list shows the further action of various reagents.

Reactions of Peptones free from albumose and purified by phosphotungstic acid.

In 5 per cent. solution, after being made noticeably alkaline with a trace of sodium carbonate.

	Fibrin antipeptone.	Fibrin amphopeptone.
Acetic acid and potassium ferrocyanide.	At first perfectly clear, later trace of opalescence.	The same.
Neutral lead acetate.	First drop, 0; more, turbidity.	The same, but much weaker.
Basic lead acetate.	Turbidity immediately; more, strong turbidity.	The same, but weaker.
Mercuric chloride.	First drop, 0; more, strong turbidity.	Turbidity immediately, growing stronger.
5 per cent. cupric sulphate.	At first clear; more, slight turbidity disappearing with great excess.	Nothing.
5 per cent. platinum chloride.	Only excess, strong turbidity.	Nothing.
Chromic acid.	Nothing.	Nothing.
Ferric chloride.	A trace gives turbidity vanishing with the least excess.	Nothing.
Glacial acetic acid and conc. sulphuric acid.	Brownish red.	The same.
Nitric acid.	The color changing yellow in the cold.	The same.
Boiling with conc. hydrochloric acid.	The color becomes slightly darker.	The same.
Millon's reaction.	At first a heavy white precipitate; on warming, dirty yellow or reddish.	The same, then beautiful red color.

Without desiring to claim especial value in general for these reactions and for smaller differences between the two peptones, some of them, however, may be more closely examined.

The slight intensification of color by boiling with concentrated hydrochloric acid is striking, for we have not ordinarily been able to obtain it, even with peptone in substance or even on the addition of

concentrated sulphuric acid. Likewise, the reaction with sulphuric acid and glacial acetic must be described as almost unsuccessful. As this, however, is nothing other than Pettenkofer's test for bile acids, for which its discoverer has recommended concentrated acetic acid as a substitute for sugar, we have also tried the reaction with sugar, without however obtaining any better result, particularly not the beautiful violet-red which albumin and the albumose bodies give. Finally, the poor result of Millon's reaction with antipeptone, in contrast to the brilliant red obtained with amphopeptone, is also to be remarked. To this reaction we shall return later.

It has already been observed by many investigators that among the products of the digestion of albumin, bodies are not infrequently met with, which give little or no lead sulphide on boiling with sodium hydroxide and lead acetate. This is not at all strange, since the peptones prepared by us show on analysis less than one per cent. of sulphur, in striking contrast to the albumins and albumose bodies, all of which contain much larger percentages. In the gland peptones (F, G, H,) the sulphur amounted to only 0·50, 0·31 and 0·57 per cent. respectively. The peculiarity of peptones in giving up a part of their sulphur when warmed with alkali, apparently stands in no direct connection with the percentage amount of sulphur. Indeed, solutions of the antipeptones G and H, of which the first possessed the lowest percentage of sulphur found, showed no browning with this test, and only a trace of it when solid particles of the peptone were heated with a concentrated solution of alkali containing lead. But the gland peptone (F) not purified by phosphotungstic acid and with only 0·15 per cent. of sulphur became slightly darkened in solution. On the other hand, the purest amphopeptone (B) with 0·77 per cent. of sulphur gave the reaction very faintly, while antipeptone C with 0·73 per cent. of sulphur gave it very plainly. Probably the reaction is not to be attributed to the peptones themselves, but proceeds from contamination with an easily decomposed substance containing sulphur, whose removal still depends on chance.

It may be assumed as completely proved that the rose or violet coloration, which the products of pancreatic digestion assume with bromine or chlorine water, is due to some special body and not to antipeptone. We had previously shown this to be the case with the antipeptone obtained by the action of trypsin from antialbumid, and have now also found in all antipeptones purified with phosphotungstic acid, the absence of all color on addition of bromine water, either in large or small quantity.

Finally, not to overlook the most striking reaction of peptones, we mention further the brilliant so-called biuret reaction which is to be seen, if possible, still more intense in the purer peptones, whatever their origin, than in those formerly used.

Composition of the Peptones.

For the sake of comparison we present in the following table the percentage composition of the various samples of peptones, calculated on the ash-free substance. The ash found, consisting in every case of calcium, a little sodium, potassium, traces of barium and iron, carbonic acid, phosphoric acid and sulphuric acid, is placed at the foot of the columns.

	Ampho- (pepsin) peptone from fibrin.			Anti- (trypsin) peptone.					
	A.	B.	b.	C.	D.	E.	F.	G.	H.
		From purified pepsin and purified with phosphotungstic acid.		Prepared from fibrin.			Glandpeptone.		
	Containing mucin-peptone.				Purified more with ether.	Purified with phosphotungstic acid.		Purified with phosphotungstic acid.	
C	44·53	48·75	48·47	47·30	47·68	46·59	44·45	42·96	44·47
H	6·49	7·21	7·02	6·73	7·03	6·69	7·17	7·26	7·15
N	16·73	16·26	16·86	16·83	16·68	18·28	17·06	17·80	17·94
S	0·72	0·77	....	0·73	....	0·67	0·50	0·31	0·57
O	31·53	27·01	....	28·41	....	27·77	30·82	31·67	29·87
Ash	8·11	3·22	2·15	5·25	10·02	3·67	5·54	1·93	2·07

The nature of the substance, which at present affords but little proof that we have to deal with a simple body and not a mixture of chemical bodies, places the greatest restrictions on the use of the above values, and only under such reserve is to be understood what is hereafter added. As regards the analysis of the single substances, we are inclined to believe that only in amphopeptone B, is there a difference in the percentage of carbon greater than the ordinary differences naturally to be expected in amorphous materials so difficult to prepare.

Amphopeptone A, with the lowest percentage of carbon, has already been described as a mixture of fibrin-peptone and mucin-peptone. If we have succeeded in removing the latter, by using

purified pepsin, then the figures obtained from *B* and *b* may be considered as representing the first gastric peptone prepared free from albumose. The correspondence between the two, in spite of the fact that by continued purification of *b* we succeeded in reducing the ash by more than 1 per cent., we think may be considered as grounds for this assumption. In opposition therefore to the majority of previous statements, including our own, which as now easily understood referred to mixtures of albumose and peptones, there is to be noticed in pure amphopeptone about 1 per cent. lower content of carbon, about as much higher a percentage of nitrogen and 0·3–0·4 per cent. lower content of sulphur.

With antipeptone, the variation from the previous results was less expected, for we are not inclined to believe that in our former long and thorough trypsin digestions, any appreciable quantity of albumose remained and in the more recent ones, the precipitate produced by ammonium sulphate was never abundant if the matter separated by boiling in a slightly acid solution was previously removed. The differences found, however, might be readily explained by the fact that the purification of the peptone had this time been more complete, owing partly doubtless to the formation of the barium compound, and partly also to the precipitation with phosphotungstic acid. The content of carbon is seen to be about 1 per cent. lower, the content of sulphur likewise lower and the percentage of nitrogen decidedly higher, in one case as much as 4 per cent. more than before. The real reason for this difference in composition appears to us to lie in the use of much larger quantities of trypsin, which formerly was only possible by using large quantities of the gland substance, so that the fibrin-antipeptone would naturally be obtained mixed with the gland peptone. It is well for the future that we know the composition of these gland peptones, for they differ essentially, in the lower content of carbon (in one case 42·96 per cent.), from all other peptones hitherto investigated. These bodies might be pronounced troublesome intruders with the same right as the mucin-peptone arising from gastric digestion, although we found in it, aside from the percentage composition, no reaction and no property which would serve to distinguish it from the other peptones.

After these considerations, little stress can be laid on the differences between the composition of pepsin and trypsin peptones. We have found, however, another difference which we will examine more closely.

Cleavage of the peptones.

As already mentioned, Millon's reaction appears very brilliant with amphopeptone, but more or less imperfectly with antipeptone. As the reaction is sure only under certain circumstances we have not neglected to perform it in every possible way, either by using the same concentration of solutions of the two peptones under exactly the same conditions, or by trying the most successful variations of the experiment for antipeptone. Thus it was found that the preparations C, D, E, and F, treated in a suitable way, gave an appreciable reaction, but in no case so that more than a dirty, generally orange red precipitate was obtained. On the contrary, all trials with gland peptone purified with phosphotungstic acid, failed to give more than a simple yellow color. Since Millon's reaction for albumin corresponds with the so-called Hoffmann's test for tyrosin, and since with albumin it probably depends on the separation of tyrosin by boiling with the acid solution of murcuric nitrate, if not by the formation of further decomposition products of tyrosin (hydroparacumaric acids) which likewise redden with the test, it might be presumed that antipeptone, in contrast to the amphopeptone of pepsin digestion, forms no tyrosin by cleavage. So far as the action of trypsin is concerned, this was already known, since the real difference between gastric and pancreatic peptones consists in the fact that only the former, when treated with trypsin, yield tyrosin together with leucin and other decomposition products—in our opinion because they contain hemipeptone capable of further cleavage (together with antipeptone). It was also known to us, however, that antipeptone during cleavage with boiling sulphuric acid yields the amido acids, and among them also tyrosin. Renewed investigations on this subject appeared called for now, since we thought ourselves in possession of much purer preparations of antipeptone.

First, we established the possibility of decomposing with trypsin, amphopeptone entirely free from albumose. A few hours' digestion in a small test tube, of 1 gram of peptone in 10 c. c. of water, containing 0·25 per cent. of sodium carbonate with a little thymol and a fragment of purified pepsin, sufficed for this purpose. By concentrating the neutralized solution and boiling the residue with alcohol, a decided residue was obtained in which balls of leucin and bundles of tyrosin were to be seen under the microscope without further preparation. The residue was also colored a beautiful violet with bromine water. We also sacrificed a large quantity of amphopeptone to the same experiment and obtained the tyrosin pure (free

from peptone) and tested it, both by Millon's reaction and Piria's test. Further, a few grams of the same preparation were heated for several days with six times its weight of sulphuric acid (2 : 3 water), and after removing the acid with barium hydroxide, tyrosin and leucin were found among the decomposition products.

Antipeptone was now likewise submitted to the above treatment. The method of conducting this experiment with albuminous bodies which we have used successfully, even with 2–3 grams of substance, is as follows. The substance is placed in a small, strong flask standing on an asbestos plate, and five or six times the weight of sulphuric acid of the above mentioned strength is added and the mixture kept boiling as long as desired. The loss of water by evaporation is prevented by connecting to the neck of the flask a glass tube 1 metre long and 1 centimetre in diameter, so that the lower end cannot be closed by a drop of water. The upper end of the tube is drawn out to a capillary point and as it does not become warm at the top during the boiling of the fluid, all loss of water is prevented.

To be as sure as possible, we have treated the antipeptone in this manner for 48 hours. The contents of the flask were then much diluted with water, filtered from the sticky residue always present, made alkaline with a concentrated solution of barium hydroxide, the barium separated from the filtrate by sulphuric acid, after which the clear fluid was concentrated and allowed to crystallize. It is not advisable to remove the sulphuric acid with barium carbonate, since the latter is unavoidably used in excess, for it is very undesirable to have the barium precipitate unnecessarily increased in bulk, as the cleavage products of albumin are difficult to remove even with hot water. Further, the mixure is made alkaline by barium carbonate and soluble barium compounds always appear, which must be removed by sulphuric acid in every case.

In the strongly concentrated solution finally obtained, in case leucin and tyrosin have really been formed, as with most albuminous bodies, their presence may be readily shown microscopically and after suitable separation of the mother liquor, or if necessary, after re-crystallization from dilute alcohol, their chemical reactions may likewise be obtained. We have succeeded after this manner in showing the presence of leucin always in antipeptone; tyrosin, however, only in a few cases, and even then only after repeated crystallization. Whenever tyrosin occurred it was in exceedingly small quantities. From the antipeptones (gland peptones H and G) with which Millon's test had hitherto failed, tyrosin could not be obtained at all, and that

tyrosin was really absent in this case was shown by the final examination of the whole united residues by Hoffman's test, which was absolutely negative, while it appeared plainly with the products of the remaining antipeptones.

Although we do not wish to consider the behavior of the gland peptones as a criterion for antipeptone in general, still this result, united with the small amount of tyrosin obtained from the latter, seemed to call for further investigations concerning the decomposition of those primary cleavage products of albumin related to the antipeptones, especially antialbumid. We began this extension of our work because during our treatment of the subject an article of Maly's* appeared, in which he described a very interesting cleavage and oxidation product, obtained by treating albumin with potassium permanganate, which product possesses the essential properties of the albumins, and yet on further decomposition does not yield tyrosin. It is questionable, therefore, whether this property is not the one directly distinguishing the bodies of the anti-group from the primary cleavage products of the albumins.

A few preliminary experiments were made with samples of antialbumid prepared by the action of boiling dilute sulphuric acid, both on fibrin and Thiry's neutralization precipitate from egg-albumin, also with the antialbumid remaining from the digestion of fibrin with trypsin, and finally with a small neutralization precipitate of so-called parapeptone from an incomplete pepsin digestion of fibrin, which we regarded as antialbumose. After these experiments as a whole, had resulted contrary to our expectations, in that a moderate amount of tyrosin appeared after boiling the substance for a long time with sulphuric acid, we submitted to decomposition a preparation from which we thought we could expect a decisive result. This preparation was an antialbumid from egg albumin, made in one of our former investigations as follows: White of egg freed from membrane, was coagulated by heat in an acid solution, the coagulum filtered, thoroughly washed and then heated for a long time at 100° C. with dilute sulphuric acid, the residue filtered, washed thoroughly with water, dissolved in sodium carbonate, precipitated by neutralization, the precipitate dissolved in 0·2 per cent. hydrochloric acid and the antialbumid freed from all other other albuminous bodies by long continued digestion with pepsin. The antialbumid was then separated from the solution by neutralization, washed, dissolved in 0·5 per

* Wiener Acad. Sitzungsber., xci, Abth. 5, February, 1885.

cent. sodium carbonate, warmed with trypsin at 40° C., and after the antialbumid had partially separated as a jelly-like mass, it was filtered and washed with water, finally with alcohol and ether. This product could not possibly contain any known albumin, albumose or peptone and undoubtedly formed the purest sample of antialbumid yet prepared.

By decomposing this body with sulphuric acid, a residue was finally obtained which to our surprise gave but the slightest reaction for tyrosin with Millon's and Hoffmann's test, with Piria's test no reaction whatever, and in spite of endeavors continued for weeks not a single crystal of tyrosin could be detected. Leucin was found in very small quantity and in addition there were seen large lustrous balls of crystals of some nitrogenous substance, too small in quantity to be identified.

Heidelberg, New Haven, } December, 1885.

On the dehydration of Glucose in the Stomach and Intestines. By R. H. Chittenden.

In a series of interesting communications* "On the physiology of the carbohydrates in the animal system," Dr. F. W. Pavy has brought forward evidence to show that glucose, generally considered as the final product of amylolytic action, can be converted within the animal body into a product of less cupric oxide-reducing power; that there exists particularly in the stomach and intestines of rabbits, a ferment which has a dehydrating action upon glucose or dextrose, transforming it into a body akin to maltose in reducing power.

Hitherto, it has been generally supposed that the transformations which carbohydrates undergo in the animal system are in the nature of gradual hydration changes, in which each step forward toward the final product is attended with the formation of bodies of increased cupric oxide-reducing power. Dr. Pavy's results, however, would tend to show that transformations in the opposite direction do occur and this notably in the stomach and intestines of rabbits.

Dr. Pavy's conclusions concerning this dehydration of glucose in the animal system, are based upon changes in the cupric oxide-reducing power of the carbohydrate, after contact with portions of the stomach and intestines for short periods of time at 48·8° C. It is a well known fact that the reducing power of pure glucose is not affected by boiling with dilute sulphuric acid, while under like treatment, maltose and similar bodies are readily converted into glucose or into a body of like cupric oxide-reducing power.

Dr. Pavy finds, as the result of a large number of experiments, that a solution of glucose or grape sugar, by mere contact with the stomach and intestines of a rabbit at 48·8° C. is changed into a body of less cupric-oxide reducing power, and that by boiling with dilute sulphuric acid this product is carried back again into glucose. Thus, in one experiment, 0·138 gram of glucose in contact with strips of stomach from a rabbit for one hour and a half at 48·8° C. showed, after removal of the dissolved albumin by boiling with sodium sulphate, a reducing power calculated to the entire amount equivalent to only 0·080 gram of glucose; while after boiling with dilute sul-

* Chemical News, 1884, vol. xlix, pages 128, 140, 155, 162, 172 and 183.

phuric acid (the solution containing two per cent. H_2SO_4) the cupric oxide-reducing power was increased to the equivalent of 0·134 gram of glucose, or nearly equal to the amount started with.

It is to be noticed in the experiment just quoted, that the cupric oxide-reducing power, before and after treatment with sulphuric acid, stand to each other in the proportion of 58 : 100, or in about the relation of maltose (61) to glucose (100). In some experiments, however, the reducing power before boiling with dilute acid, was so low as to warrant the belief that dextrins were also formed. This result is a type of many similar ones obtained by Pavy with the stomach and intestines from various animals and in no instance, in the case of rabbits at least, so far as reported, were negative results obtained.

The discovery of such a dehydrating ferment, hitherto unsuspected, appeared to be a matter of so much importance that experiments have been tried in this laboratory from time to time during the past two years, with the view of confirming in part at least some of Dr. Pavy's results. To our surprise, however, in no case, have we been able to obtain results corresponding to those of Pavy's, although the animals experimented with (rabbits and cats) were taken in various stages of digestion. We therefore record here, some of the results simply in the hope that some light may be thrown upon the cause of this discrepancy; or if, as may be, the ferment is not invariably present, some reason may be found for its constant absence in the tissues of the animals experimented with, and thus light be thrown upon the conditions which control its presence.

The glucose used in the following experiments was a sample of crystallized anhydrous glucose presented to the laboratory by Dr. Arno Behr. The sugar was quite pure, as was ascertained by testing both its reducing power and specific rotary power, and more important still, was not at all affected by boiling with dilute sulphuric acid. Thus 50 c. c. of a one per cent. solution of the glucose, mixed with sufficient 10 per cent. sulphuric acid to have the mixture contain two per cent. of H_2SO_4, was boiled for one and one-half hours, the flask being connected with an inverted Liebig's condenser to prevent concentration. The solution was then neutralized, diluted to 100 c. c. and tested with Fehling's solution according to the method of Allihn. 25 c. c. yielded 0·2414 gram of metallic copper, corresponding to 0·1246 gram of glucose, whereas the 25 c. c. of solution should have contained 0·1250 gram of sugar. Evidently then, the reducing power of the sugar is not affected by treatment with dilute acid.

First experiment.—A rabbit in full digestion was killed, the stom-

a ch emptied of its contents and then divided into two longitudinal halves along the curvatures. One-half, after being cleansed, was finely divided and placed in a small beaker with 70 c. c. of water containing 0·200 gram of glucose. An equivalent amount of the small intestine, similarly cleaned and divided, was placed in a second beaker in contact with 70 c. c. of water containing 0·150 gram of glucose. In both cases the entire walls, including *muscularis* and *mucosa*, were taken, since Pavy has indicated that the converting principle is situated not on the surface of the mucous membrane, but in the deeper part. The two mixtures were then placed in a bath and warmed at 48·8° C. for nearly two hours; after which they were boiled, crystals of sodium sulphate being added to aid the removal of the dissolved albumin. The individual filtrates and washings were concentrated and finally brought to a volume of 100 c. c. Of this, 25 c. c. were used to determine the cupric oxide-reducing power of the solution directly, while 50 c. c. of each solution were mixed with sufficient ten per cent. sulphuric acid to insure a content of two per cent. and then boiled for two hours, in connection with an inverted Liebig's condenser to prevent concentration. The acid solutions were then neutralized, concentrated somewhat and finally brought back to a volume of 50 c. c. Following are the analytical results obtained with the two solutions, the reducing power being determined by Allihn's gravimetric method.*

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0746 gram Cu=0·0381 gram dextrose ×4=0·1524 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0728 gram Cu=0·0372 gram dextrose ×4=0·1488 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0512 gram Cu=0·0265 gram dextrose ×4=0·1060 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0520 gram Cu=0·0269 gram dextrose ×4=0·1076 gram dextrose.

Here, there is no evidence whatever that the glucose was affected by its two hours' contact with the stomach and intestine of the rabbit at 48·8° C., the temperature specified by Pavy as that best adapted for the reaction. Certainly the reducing power of the glucose solution is essentially the same before and after treatment with sulphuric

* Zeitschrift für Analytische Chemie, 22. Jahrgang, p. 448.

acid. Somewhat in accord with Pavy's results, however, is the fact that while 200 milligrams of glucose were introduced into the stomach mixture and 150 milligrams with the intestines, only 152·4 milligrams were recovered from the former and 106 milligrams from the latter, although the residues after heating with sodium sulphate, were repeatedly and thoroughly washed with hot water. Assuming that this loss of sugar in the two cases is really due to change of glucose into lower reducing bodies, the relative reducing power of the sugar before and after contact with the stomach and intestines would be 100 : 76·2 and 100 : 70·6 respectively. But if there had been any such change in reducing power, the treatment with sulphuric acid would certainly have indicated it.

Second experiment.—A rabbit in full digestion was killed and half of the stomach and a portion of the small intestine were cleaned and finely divided. The stomach tissue was then heated at 48·8° C. for one and one-half hours, with 70 c. c. of water containing 0·200 gram of glucose and the portion of intestine for the same length of time, with a like amount of glucose. Treated then in the same manner as the preceding solutions, the following results were obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0901 gram Cu =0·0460 gram dextrose ×4=0·1840 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0882 gram Cu =0·0450 gram dextrose ×4=0·1800 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0690 gram Cu =0·0353 gram dextrose ×4=0·1412 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0726 gram Cu =0·0371 gram dextrose ×4=0·1484 gram dextrose.

In this experiment, a larger amount of sugar was recovered in the case of the stomach tissue than in the preceding experiment, but in neither the stomach or intestine is there any evidence of change in the reducing power of the sugar before and after treatment with sulphuric acid. In this connection it is to be remembered, that the only ground for belief in the existence of a dehydrating ferment in the stomach is the change, noticed by Pavy, in the reducing power of the sugar under the above method of treatment. Our method of treatment with sulphuric acid, moreover, both as to the length of time the mixtures were heated and the strength of acid employed, was in accord with the method used by Pavy. In addition, the same

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method of treatment was applied to a known solution of maltose with satisfactory results, viz.: a rapid and complete change into dextrose as attested by the proper proportional increase in reducing power. Furthermore, we are led to infer from Pavy's results that the action of the ferment is to be seen to the best advantage in the rabbit. Thus Pavy states,* that without having made any precise comparative observations, 'I am under the impression that the stomach and the intestine of the rabbit act more energetically than the stomach and intestine of the other animals I have tried. It also appears to me that the stomach acts more energetically than the intestine, and in some instances I have noticed that the effect produced, has stood in relation to the amount of ferment material used." The latter half of this statement would tend to indicate that the main reason for our not recovering all of the glucose is to be found either in a lack of sufficient washing of the tissue residue at the end of the experiment, or else in a slight fermentation by which a portion of the sugar might be decomposed; for as is to be noticed in nearly all of the experiments recorded here, far less sugar is lost in the stomach than in the intestine, whereas if due to change in reducing power from the action of a dehydrating ferment, the greatest loss, Pavy's statement being correct, would be observed by contact with the stomach tissue. On the contrary, our results show greatest loss in the intestine, which if due to mechanical reasons would be naturally explained, since the glairy mass of tissue, even after boiling, affords mechanical obstacles to a thorough extraction. That this is doubtless the true explanation, in part at least, is evidenced by the fact that a portion of the stomach or intestine, previously boiled with water to destroy its vitality, yields results after the same order as those already given, except that the amount of sugar recovered is greater, as would naturally be expected since the tissue being already coagulated would not enclose the sugar so completely. Thus, on warming one-half of a rabbits' stomach, previously divided and boiled with water, with 0·200 gram of glucose for two hours, there was recovered 0·1910 gram of the glucose; while from a portion of the small intestine, likewise boiled and treated with the same amount of glucose, there was recovered only 0·1840 gram of the sugar. Furthermore, fermentation of the sugar would naturally occur more quickly in the intestines than in the more compact stomach tissue. Be that as it may, the reducing power of neither solution was affected by boiling with dilute sulphuric acid.

* Chemical News, vol. xlix, p. 141.

Third experiment.—A rabbit in a condition of hunger was killed, the stomach divided longitudinally along the curvatures, and one-half after being cleaned and finely divided, was placed in contact with 0·200 gram of glucose dissolved in 70 c. c. of water, and warmed at 48·8° C. for one and one-half hours. The mixture was then heated to boiling with the addition of some crystals of sodium sulphate, the tissue and coagulated albumin filtered off and the residue washed with about 300 c. c. of hot water. The fluid was concentrated, brought to a volume of 100 c. c. and then treated as in the preceding experiments. Following are the results obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0834 gram Cu = 0·0425 gram dextrose × 4 = 0·1700 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0798 gram Cu = 0·0407 gram dextrose × 4 = 0·1628 gram dextrose.

Here, as before, there is no evidence of any change in the character of the glucose; still in spite of the comparatively large volume of wash-fluid used, the sugar was not wholly recovered.

Fourth experiment.—A cat killed in full digestion was employed in this experiment. One-half of the stomach, finely divided, was placed in contact with 0·200 gram of glucose in 75 c. c. of water. A portion of the small intestine was also treated with a like amount of sugar, in the same manner. Both were warmed for three hours at 48·8° C., then treated by the same method as used in the preceding experiments.

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0879 gram Cu = 0·0449 gram dextrose × 4 = 0·1796 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0886 gram Cu = 0·0452 gram dextrose × 4 = 0·1808 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0668 gram Cu = 0·0342 gram dextrose × 4 = 0·1368 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0684 gram Cu = 0·0350 gram dextrose × 4 = 0·1400 gram dextrose.

Here again, there is no evidence whatever of any change in the reducing power of the sugar solution.

Pavy has also pointed out that the stomach and intestine of the rabbit, as well as of other animals, have a transformative action on saccharose as well as on dextrose. The transformative energy how-

ever of the intestine, is much greater in this case than that of the stomach; which, according to Pavy, accounts for the early discovery by Bernard of the well known action of the intestine on cane sugar. Bernard supposed dextrose to be formed, but Pavy shows that maltose or a body resembling maltose in reducing properties, is the usual product and that glucose or dextrose is formed only in the presence of considerable ferment. The ferment which produces this change, unlike the ferment which acts upon glucose, is situated on the surface of the mucous membrane and thus frequently the contents of the stomach are likewise found to possess transformative power.

Fifth experiment.—A rabbit with stomach partially filled with food was killed, the stomach rinsed with water, then minutely divided and separated into two equal parts. One portion was placed in contact with 0·195 gram of glucose in 75 c. c. of water, while the other portion was mixed with a like amount of pure saccharose, also in 75 c. c. of water. A portion of the small intestine was likewise finely divided and one portion placed in contact with 0·195 gram of glucose in 75 c. c. of water and the other portion with 0·200 gram of saccharose dissolved in 75 c. c. of water. All four mixtures were warmed at 48·8° C. for two hours, then heated to boiling with the addition of sodium sulphate and finally each brought to a volume of 100 c. c. 25 c. c. of the saccharose solution, which had been in contact with the stomach tissue, gave no reduction whatever with Fehling's solution. 25 c. c. of the saccharose-intestine solution, however, gave 0·0726 gram Cu, equivalent to 0·0371 gram dextrose.

With the glucose solutions, the following results were obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0906 gram Cu = 0·0462 gram dextrose × 4 = 0·1848 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0889 gram Cu = 0·0454 gram dextrose × 4 = 0·1816 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0712 gram Cu = 0·0364 gram dextrose × 4 = 0·1456 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0689 gram Cu = 0·0353 gram dextrose × 4 = 0·1412 gram dextrose.

With glucose, the same results are to be observed here as in the preceding experiments; the only variations in reducing power, before and after treatment with sulphuric acid, being such as would come within the ordinary limits of error. In one single case, a transforma-

tion of saccharose was noticed when the sugar solution was warmed for two hours with a portion of stomach tissue from a rabbit killed in full digestion. The reduction with Fehling's solution was quite strong.

With glucose, however, many experiments have been tried in addition to those given above, and invariably with the same negative result. The conditions of the experiments, moreover, are in many cases identical with those of Pavy's except in the method of determining reducing power. There seems, therefore, to be no plausible explanation of the results obtained, other than that in the above experiments there was no dehydrating ferment present. Pavy states that the ferment in question, or rather "the active principle concerned in the transformation of glucose is susceptible of being destroyed by the agency of gastric digestion," so that there is the possibility of such destructive action having taken place in the stomach of the animals experimented with. It is further stated, however, that the converting principle is situated in the underlying portion of the mucous membrane, so that destruction could hardly be expected, except perhaps in the slow self-digestion occurring after death. Certainly, the carefully rinsed tissue could not have retained sufficient gastric juice to affect the results. Furthermore, such decomposition would apply only to the stomach mixture and not to the intestines, unless sufficient proteolytic ferment from the pancreatic juice should adhere to the walls of the intestines to exert destructive action; but in the last experiment given, it is to be noticed that the saccharose ferment, which is presumably equally sensitive, showed vigorous action while the glucose was unaffected.

It seems strange, therefore, if such a dehydrating ferment is normally present in the alimentary tract, that we have not been able to obtain some tangible evidence of its presence, either in the stomach or intestines.

Since the above was written, the writer has noticed that M. Ogáta,* experimenting with dogs, has also been unable to confirm Pavy's results, both in the dehydration of glucose and in the inversion of saccharose.

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*See Jahresbericht für Thierchemie, xv, 275.

Influence of Uranium Salts on the Amylolytic Action of Saliva and the Proteolytic Action of Pepsin and Trypsin. By R. H. Chittenden and M. T. Hutchinson, Ph.B.

Little is known regarding the physiological, or even toxical action of the uranium salts. In 1825, Gmelin* reported upon the results of some experiments on the toxic action of uranic nitrate, but aside from the work done at that time, little is known regarding the action of uranium. It is our purpose, therefore, to carry out in this Laboratory, as opportunity offers, a series of experiments on the physiological and toxical action of uranium salts, and we have commenced the work by endeavoring to ascertain the influence of the above salts on the amylolytic and proteolytic action of the ferments occurring in the digestive fluids of the body. In this connection we wish to express our obligations to Professor H. Carrington Bolton, of Trinity College, for his kindness in supplying us with an abundance of chemically pure uranium compounds.

1. *Influence on the amylolytic action of saliva.*

The method employed in determining the extent of amylolytic action was much the same as that previously† used by one of us, except that the amounts of reducing substances formed under the different conditions of the experiments, were determined volumetrically by Fehling's solution, instead of by Allihn's gravimetric method. The experiments were made in series, in which one digestion of each series served as a control for comparison.

The volume of each digestive mixture was 100 c. c. and contained 1 gram of perfectly pure potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva and a given percentage of the uranium salt to be experimented with. The mixtures were then warmed at 40° C. for 30 minutes, at the end of which time, further ferment action was stopped by heating the solutions to boiling. The saliva employed in the experiments was human mixed saliva, freshly collected, filtered and made as neutral as possible with 0·2 per cent. hydrochloric acid, then diluted with water in the pro-

* Edinb. Med. Surg. Gaz., xxvi., 136.

† Studies from this Laboratory, vol. i, 1884–5, p. 2 and 53.

portion of 1 : 5. Hence, each digestive mixture contained 2 c. c. of undiluted saliva.

The amount of reducing substances, which for the sake of convenience are calculated as dextrose, were, as already mentioned, determined volumetrically and from the data so obtained, the percentage of starch converted was likewise calculated.

Uranyl nitrate.

With this salt the following results were obtained :

$UO_2(NO_3)_2 + 6H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4135 gram.	37·21 per cent.	100·0
0·0001 per cent.	0·4083	36·74	98·7
0·0008	0·3878	34·85	93.6
0·0005	0·3698	33·28	89·4
0·001	0·3612	32·50	87·3
0·003	0·3131	28·17	75·5

The inhibitory action of the uranyl salt is plainly manifest in these results. A second series of experiments, with still larger percentages of uranyl nitrate, show the retarding action still more plainly.

$UO_2(NO_3)_2 + 6H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4066 gram.	36·59 per cent.	100·0
0·001 per cent.	0·4000	36·00	98·3
0·002	0·3880	34·92	95·4
0·003	0·3034	27·30	74·6
0·004	0·2545	22·90	62·5
0·005	0·1550	13·99	38·2
0·008	trace.		

The largest percentage of the salt used (0·008 per cent.), is seen to almost entirely prevent the action of the ferment, thus showing how extremely sensitive the salivary ferment is to the action of this salt. Comparing the two series of experiments, it is seen further, that a given percentage of the salt, say 0·001 per cent., is much more active in one case than in the other, indicating that the action of the salt is not constant. This is undoubtedly true to a limited extent. The action of a given percentage of the salt is constant only under *like conditions.* In the above series of experiments, the saliva is different in the two cases, and the real explanation of the difference in action is to be sought for in the amount of proteid matter contained in the saliva. Undoubtedly the retarding action of the uranium salt is checked, in part at least, like that of mercuric chloride,* by the

* Studies from this Laboratory, 1884–85, p. 71.

proteid matter of the saliva. Uranium is a well-known precipitant of albuminous matter and hence the larger the amount of albumin and globulin contained in the saliva, the weaker the retarding action of the uranium salt. Previous experiments have shown that saliva varies somewhat from day to day in its content of proteid matter, as well as in the amount of ferment, and although the former difficulty is obviated as much as possible by diluting the saliva, still this point must be borne in mind in making comparisons of the different series of experiments.

Uranyl acetate.

This salt appeared somewhat more inhibitory in its action than the nitrate, due possibly to its greater acidity. The two following series show the extent of action:

$UO_2(C_2H_3O_2)_2 + H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4049 gram.	36·44 per cent.	100·0
0·001 per cent.	0·2805	20·74	56·9
0·002	0·1698	15·28	41·9
0·003	trace.		
0	0·4082	36·28	100·0
0·0003	0·4331	38·97	107·3
0·0005	0·3322	29·89	82·4
0·0008	0·3192	28·89	79·6
0·0010	0·2882	25·98	71·4

The presence of 0·003 per cent. of the salt almost entirely stops the action of the ferment, while 0·0003 per cent. decidedly increases amylolytic action. This latter influence is similar to that exerted by many other metallic salts in very small fractions of one per cent. and is doubtless to be attributed, in part at least, to the stimulating action of the acid-proteids formed, or in part, as suggested by Duggan,* to a more complete neutralization of the digestive fluid.

Ammonio uranous sulphate.

With this salt the following results were obtained:

$U(SO_4)_2 + (NH_4)_2SO_4 + H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·3384 gram.	30·45 per cent.	100·0
0·0003	0·3935	35·41	116·3
0·0005	0·3798	34·18	112·2
0·0008	0·3563	30·40	99·8
0·001	0·3550	30·28	99·4
0·002	0·2951	26·55	87·2
0·003	0·0805	7·24	23·7

* See Amer. Chem. Jour. vol. viii, p. 211.

Here there is to be seen both stimulating and inhibitory action, both quite pronounced; and further, the salt is perfectly neutral, so that such action as is exerted must be due to the salt itself.

Sodio uranic sulphate.

This salt, which like the preceding was exactly neutral to test papers, shows both stimulating and retarding action, but in extent somewhat smaller than that of the uranous salt. Following are the results obtained:

$UO_2SO_4 + Na_2SO_4$.	Total amount reducing bodies.	Starch converted	Relative amylolytic action.
0	0·4049 gram.	36·44 per cent.	100·0
0·0003 per cent.	0·4100	36·90	101·3
0·0005	0·4000	36·00	98·8
0·0008	0·4100	36·90	101·2
0·001	0·4117	37·05	101·7
0·002	0·2530	22·77	62·5
0·003	0·2000	18·00	49·4
0·005	trace.		

Potassio uranic oxychloride.

$UO_2Cl_2 + 2KCl + 2H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4032 gram.	36·26 per cent.	100·0
0·0005 per cent.	0·3951	35·55	98·0
0·0008	0·4016	36·14	99·6
0·001	0·4083	36·74	101·3
0·002	0·1881	16·92	46·6
0·003	0·1073	9·65	26·6
0·005	trace		

This salt had an acid reaction and its retarding effects are seen to be somewhat more pronounced than that of the two preceding neutral salts.

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4135 gram.	37·21 per cent.	100·0
0·0003 per cent.	0·4298	38·68	103·9
0·0005	0·4016	36·14	97·1
0·0008	0·4049	36·44	97·9
0·001	0·3873	34·85	93·6
0·002	0·4016	36·14	97·1
0·003	0·3843	33·86	89·6

$(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4117 gram.	37·05 per cent.	100·0
0·004 per cent.	0·2378	21·40	57·7
0·005	0·2144	19·29	52·0
0·006	0·2316	21·39	57·7
0·007	0·1845	16·60	44·8
0·008	0·1765	15·88	42·8
0·010	trace		

The action of this salt is mainly a restraining one, but the action is pronounced only with the larger percentages.

As to the way in which these uranium salts diminish the amylolytic action of the ferment, we cannot say definitely. What has previously* been written regarding the action of other metallic salts, under like conditions, is doubtless true here. Loss of amylolytic power is due in part, no doubt, to partial direct destruction of the ferment, as well as to change in the reaction of the fluid. Coupled with this destructive action, however, there must be in addition something in the mere presence of these salts, dependent on chemical constitution, that controls the action of the ferment.

The following table shows the relative acceleration and retardation of the various salts, compared with their respective controls expressed as 100.

2. *Influence on the proteolytic action of pepsin-hydrochloric acid.*

The influence of uranium salts on the proteolytic action of pepsin-hydrochloric acid, was determined by ascertaining the amount of fibrin digested or dissolved in a given time, by a definite volume of a standard, artificial gastric juice, in the presence of varying amounts of the uranium salts. The gastric juice was made by dissolving 10 c.c. of a glycerin extract of pepsin in one litre of 0·2 per cent. hydrochloric acid. The volume of each digestive mixture was 50 c.c.; composed of 25 c.c. of the above mentioned artificial gastric juice and 25 c.c. of 0·2 per cent. hydrochloric acid, containing the necessary amount of uranium salt. The proteid material consisted of purified fibrin, coarsely powdered and dried at 100° C. One gram of fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for one hour and then the undissolved residue was collected on weighed filters and finally dried at 100–110° C. until of constant weight. The amount of fibrin dissolved is taken as a measure of the proteolytic action.

* See Studies from this Laboratory, vol. i, 1884–5, pp. 70–75.

TABLE SHOWING RELATIVE ACCELERATION AND RETARDATION OF AMYLOLYTIC ACTION.

Percentage of Salts....	0·0001	0·0003	0·0005	0·0008	0·001	0·002	0·003	0·004	0·005	0·006	0·007	0·008
Uranyl nitrate.....	98·7	93·6	89·4	.....	87·8	.....	75·5	62·5	38·2	.....	.....	0
Uranyl acetate.....	.....	107·3	82·4	79·6	71·4	41·9	0	.....	.....	.....	.....	.....
Ammonio uranous sulphate.....	.....	116·3	112·2	99·8	99·4	87·2	23·7	.....	.....	.....	.....	.....
Sodio uranic sulphate..	.....	101·3	98·8	101·2	101·7	62·5	49·4	.....	0	.....	.....	.....
Potassio uranic oxychloride.....	.....	.....	98·0	99·6	101·3	46·6	26·6	.....	0	.....	.....	.....
Ammonio uranic citrate.....	.....	103·9	97·1	97·9	93·6	97·1	89·6	57·7	52·0	57·7	44·8	42·8

Following are the results obtained with the various salts:

Uranyl nitrate.

$UO_2(NO_3)_2 + 6H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1353 gram.	86·47 per cent.	100·0
0·025 per cent.	0·1365	86·35	99·8
0·050	0·1378	86·22	99·7
0·100	0·2397	76·03	87·9
0·500	0·5003	49·97	57·8
1·000	0·6638	33·62	38·8

Uranyl acetate.

$UO_2(C_2H_3O_2)_2 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1458 gram.	85·47 per cent.	100·0
0·025 per cent.	0·1581	84·19	98·5
0·050	0·1867	81·33	95·1
0·100	0·2052	79·48	92·9
0·500	0·7507	24·93	29·2
1·000	1·0050	0	0

It is to be noticed here, that the retarding action of the acetate, as with saliva, is far greater than the nitrate, a fact which is doubtless dependent in this case on the nature of the acid united with the uranium. Further, it is to be noticed, that the action of uranyl sulphate falls about midway between the action of the nitrate and acetate. These facts accord with views previously* expressed, and show plainly that the extent of the retarding action of salts in general is dependent in part, on the liberation of the acid of the salt and the *digestive power* of the pepsin-*acid* formed. Experiments have shown that nitric acid of appropriate strength, united with pepsin, is about four-fifths as active as hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as hydrochloric of the same strength and that acetic acid is practically inactive. Hence, the base being the same, acetates, citrates, and other salts, the acids of which are not capable of working with pepsin will most readily retard gastric digestion. This view being correct, uranyl nitrate, sulphate and acetate should retard gastric digestion in just such relative proportion as our experiments show they actually do.

* See Studies from this Laboratory, 1884–85, p. 94–95.

Uranyl sulphate.

$UO_2SO_4+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1832 gram.	81·68 per cent.	100·0
0·025 per cent.	0·2545	74·55	91·3
0·050	0·2673	73·27	89·7
0·100	0·3155	68·45	83·8
0·500	0·6084	39·16	47·9
1·000	0·8225	17·75	21·7

Ammonio uranous sulphate.

$USO_4+(NH_4)_2SO_4+H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1833 gram.	81·67 per cent.	100·0
0·025 per cent.	0·2859	71·41	87·4
0·050	0·3143	68·57	83·9
0·100	0·3742	6·258	76·6
0·500	0·9113	8·87	10·8
1·000	1·0070	0	0

A comparison of the action of the two last salts, shows plainly that the ammonio uranous compound has a far greater inhibitory action than the simple uranyl sulphate.

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2+(NH_4)_3C_6H_5O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1747 gram.	82·53 per cent.	100·0
0·025 per cent.	0·1795	82·05	99·4
0·050	0·2102	78·98	95·7
0·100	0·2180	78·20	94·7
0·500	0·9055	9·45	11·4
1·000	0	0	0

Sodio uranic sulphate.

$UO_2SO_4+Na_2SO_4+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2624 gram.	73·76 per cent.	100·0
0·025 per cent.	0·2666	73·34	99·4
0·050	0·3688	63·12	85·5
0·100	0·4438	55·62	75·6
0·500	0·8131	19·69	26·7
1·000	0·9891	1·09	1·5

In the two last series, the ammonio uranic citrate is noticeable for not causing a gradual diminution in the proteolytic action of the ferment; but on the contrary, it gives rise to a sudden and rapid falling off in proteolytic action, when a certain percentage of the salt is added. The same thing is to be noticed in the case of the uranyl

acetate and the reason doubtless lies in the fact that the acid in these two salts is wholly incapable of forming an active compound with pepsin, and thus when a percentage of the salt is added sufficient to use up all of the hydrochloric acid of the gastric juice, digestive action comes to a full stop.

Potassio uranic oxychloride.

$UO_2Cl_2 + 2KCl + 2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3063 gram.	69·37 per cent.	100·0
0·025 per cent.	0·2472	75·28	108·0
0·050	0·2123	78·77	113·5
0·100	0·2532	74·68	107·6
0·300	0·2648	73·52	105·9
0·500	0·3578	64·22	92·5

With this salt, unlike any of the preceding, there is to be seen a direct stimulating action on the ferment. Only in the presence of 0·5 per cent. of the salt is there any retarding effect produced. This naturally suggests that possibly uranium *per se*, at least in small fractions of a per cent., has really a stimulating effect on ferment action, but that owing to its combination with acids, in the formation of salts, its apparent effects in the case of pepsin-hydrochloric acid are those due to combination of the normal acid of the gastric juice and liberation of the acid of the uranium salt. In this way only, can we explain the noticeable difference in action of the oxychloride and the other uranium salts. Thus 0·5 per cent. of the former causes but slight diminution in proteolytic action, while with all the other salts, the same percentage causes on an average, a diminution in proteolytic action of at least 50 per cent. This would apply, of course, only to small percentages of uranium, for larger amounts of oxychloride would cause the formation of an indigestible uranium-albumin compound.

Here, however, as in the case of all the salts, the action of any given percentage is constant only under definite conditions. Diminish the amount of ferment, for example, and the amount of dissolved proteid matter consequent thereto, and then the retarding action of the same percentage of uranium salt will be correspondingly increased.

This is well illustrated in the following series of experiments with potassio uranic oxychloride. Using the same percentages of salt as employed in the preceding series, with the same strength of acid, but with only half the same content of pepsin extract, and the following results were obtained:

Uranium salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1652 gram.	83·48 per cent.	100·0
0·025 per cent.	0·1982	80·18	96·0
0·050	0·2192	78·08	93·5
0·100	0·2569	74·31	89·2
0·300	0·3486	65·14	78·0

Increasing the percentages of the oxychloride still further, either with this strength of pepsin or the preceding, and there is seen a gradual diminution in the action of the ferment. Compared, however, with the action of the preceding salts, retardation is seen to be quite slow; thus even 2·0 per cent. causes a diminution of proteolytic action amounting to only 50 per cent.

The following table of comparisons shows the relative acceleration and retardation of the various salts compared with their respective controls expressed as 100.

TABLE SHOWING RELATIVE PROTEOLYTIC ACTION.

Percentage of Salts	0·025	0·05	0·1	0·8	0·5	1·0	2.0
Uranyl nitrate	99·8	99·7	87·9	....	57·8	38·8	....
Uranyl acetate	98·5	95·1	92·9	....	29.2	0	....
Uranyl sulphate	91·3	89·7	83·8	....	47·9	2·17	....
Ammonio uranous sulphate	87·4	83·9	76·6	....	10·8	0	....
Sodio uranic sulphate	99·4	85·5	75·6	....	26·7	1·5	....
Ammonio uranic citrate	99·4	95·7	94·7	....	11·4	0	....
Potassio uranic oxychloride 1	108·0	113·5	107·6	105·9	92·5	..	....
Potassio uranic oxychloride 2	96·0	93·5	89·2	78·0	81·0	66·2	49·4

In retarding proteolytic action, the uranium salts act in part by combining with the proteid matter to be digested, forming a uranium-albumin compound, which is indigestible. Further, in a solution at all concentrated, the uranium salt is liable to precipitate mechanically a portion or all of the pepsin along with the albuminous matter. In addition to this, however, retardation is also due, as already expressed, to liberation of the acid of the salt by the hydrochloric acid of the gastric juice and to the subsequent formation of a pepsin-acid only partially, or not at all, capable of digestive action.

3. *Influence on the proteolytic action of trypsin.*

The method employed in determining the extent of proteolytic action in this case was much the same as in the preceding. The trypsin solution was made as neutral as possible and was prepared from dried ox pancreas, previously extracted with alcohol and ether; 20 grams dry pancreas, extracted with 200 c.c., 0·1 per cent. salicylic acid and ultimately diluted to 1 litre. A little thymol was added to prevent decomposition. 50 c.c. of the trypsin solution were used in each experiment, together with 1 gram of prepared fibrin and the necessary amount of uranium salt.

The first experiment was tried with uranyl nitrate, the mixtures being warmed at 40° C. for six hours. Following are the results:

$UO_2(NO_3)_2 + 6H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2927 gram.	70·73 per cent.	100·0
0·010 per cent.	0·3328	66·72	94·3
0·025	0·3460	65·40	92·4
0·050	0·4198	58·02	82·0
0·100	0·5004	49·96	70·6
0·500	———	0	0

With this salt retarding action is seen to be gradual up to a certain point, and then suddenly all ferment action ceases.

Uranyl acetate.

$UO_2(C_2H_3O_2)_2 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4284 gram.	57·66 per cent.	100·0
0·010 per cent.	0·4591	54·09	93·8
0·025	0·5460	45·40	78·7
0·050	0·6094	39·06	67·7
0·100	0·8173	18.27	31·6
0·500		0	0

This series of experiments was warmed at 40° C. for about five hours. The inhibitory action of the salt is seen to be more pronounced than that of the nitrate; indeed, there is to be seen here, the same difference in action noticed in the case of the amylolytic ferment.

Uranyl sulphate.

With this salt, under exactly the same conditions of time and temperature as the preceding, the following results were obtained:

$UO_2SO_4+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3790 gram.	62·10 per cent.	100·0
0·010 per cent.	0·3863	61·37	98·8
0·025	0·5023	49·77	80·1
0·050	0·6066	39·34	63·3
0·100	0·8083	19·17	30·0
0·500	— —	0	0

These results are seen to accord almost exactly with the preceding and show that both salts have an action on this ferment much more pronounced than on pepsin-hydrochloric acid.

Ammonio uranous sulphate.

$U(SO_4)+(NH_4)_2SO_4+H_2O$	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4025 gram.	59·75 per cent.	100·0
0·010 per cent.	0·4031	59·69	99·7
0·025	0·4229	57·71	96·4
0·050	0·4824	51·76	86·6
0·100	0·5743	42·57	72·9
0·500	——	0	0

Sodio uranic sulphate.

$UO_2SO_4+Na_2SO_4+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3261 gram.	67·39 per cent.	100·0
0·010 per cent.	0·3414	65·86	97·4
0·025	0·3588	64·12	94·8
0·050	0·3961	60·39	89·5
0·100	0·4462	55·38	82·1
0·500	——	0	0

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2+(NH_4)_3C_6H_5O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3955 gram.	60·45 per cent.	100·0
0·010 per cent.	0·4934	50·66	83·8
0·025	0·5282	47·18	78·0
0·050	0·6618	33·82	55·9
0·100	0·7705	22·95	37·8
0·500	0·7168	28·32	46·8

Potassio uranic oxychloride.

$UO_2Cl_2+2KCl+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4234 gram.	56·76 per cent.	100·0
0·010 per cent.	0·4498	55·02	95·4
0·025	0·5224	47·76	82·8
0·050	0·5629	43·71	75·9
0·100	0·7087	29·13	50·8
0·500	——	0	0

With the exception of ammonio uranic citrate, the four last salts experimented with, show about the same degree of inhibitory action; the citrate, however, appears less pronounced in its action than the others. As to the manner in which the uranium salts retard the proteolytic action of the pancreatic ferment, it is probable that the main explanation is to be found in the power possessed by the former of combining with proteid matter in general; combining with and rendering indigestible the albuminous material added to the digestive mixture and perhaps precipitating, or even destroying, the ferment itself. Further, the reason why certain salts appear less active than others is perhaps to be found in the fact that in the precipitation of albuminous matter by uranium salts, the uranium combines directly with the proteid matter, thus liberating the acid of the salt; and as trypsin is inactive in the presence of free mineral acids, and only partially active in the presence of combined acids (combined with proteids), it follows that an organic salt, such as a citrate, would naturally be less active as a retarding agent, than the nitrate or sulphate.

The following table shows the relative retardation of the various salts expressed in terms of relative proteolytic action:

TABLE SHOWING RELATIVE PROTEOLYTIC ACTION.

Percentage of Salts	0·01	0·025	0·05	0·1	0·5
Uranyl nitrate	94·3	92·4	82·0	70·6	0
Uranyl acetate	93·8	78·7	67.7	31·6	0
Uranyl sulphate	98·8	80·1	63·3	30·8	0
Ammonio uranous sulphate	99·7	96·4	86·6	72·9	0
Sodio uranic sulphate	97·4	94·8	89·5	82·1	0
Ammonio uranic citrate	83·8	78·0	55·9	37·8	46·8
Potassio uranic oxychloride	95·4	82·8	75·9	50·8	0

It is thus seen that uranium salts, in the main, like most other metallic salts, exert a decided retarding influence on the action of the digestive ferments.

The Relative Distribution of Antimony in the organs and tissues of the body, under varying conditions.

By R. H. Chittenden and Joseph A. Blake, B.A., Ph.B.

Orfila, many years ago, proved that salts of antimony, like the salts of other metallic poisons, are absorbed and can be detected in the animal tissues and secretions, especially in the liver and kidneys; and further that the absorbed antimony is slowly discharged from these quarters through the medium of the urine. These early results were confirmed by other investigators, notably Danger and Flandin and by Panizza and Kramer, the latter of whom detected antimony, not only in the urine, but also in the blood of a man during a course of tartar emetic.* Orfila's work also indicated that while the elimination of absorbed antimony commences very quickly, it is a comparatively slow process; thus in one instance he stated † that he found antimony in the fat, liver and bones of a dog that had taken, three months and a half before its death, 46·5 grains of tartar emetic during a period of ten days, and that similar results were obtained in a second case in which the interval was four months. Presumably, however, elimination is much more rapid than these figures would seem to indicate.

Dr. Richardson, however, found antimony in abundant proportions in the liver, and in smaller proportions in the kidney and heart, twenty-one days after the last dose of antimony had been taken. As to the relative distribution of absorbed antimony, the experiments of Drs. Nevins and Richardson are the only important ones recorded. Dr. Nevins,‡ experimenting on rabbits, with tartar emetic in doses of 0·5, 1 and 2 grains four times daily, found that the weakest rabbit died after taking 12 grains, the strongest after taking 72 grains of the poison. Other rabbits were killed at varying lengths of time after taking the last dose of poison (31, 14, 4, and 3 days), and in every case antimony was found in large quantity in the liver, in smaller quantities in the spleen and stomach. Antimony was likewise found in the kidneys and urine of those animals that survived for some time, also in the lungs and in those that lived 15 days, in

* See Christison on Poisons, p. 372.

† Traité de Toxicologie.

‡ See Reese, Manual of Toxicology, p. 259–260 and Woodman & Tidy, Forensic Medicine and Toxicology, p. 128–129.

the bones likewise. Dr. Nevins further states, that it was difficult to detect the poison in the muscles and in the blood, but it was found in the bones as late as the thirty-first day after discontinuing the poison.

Dr. Richardson* in 1856, examined the tissues of a dog that died 1 hour and 40 minutes after a solution containing a drachm of tartar emetic had been injected into the cellular tissue. The antimony was found in the following parts, in the order given as to quantity; blood, vomit, rectum, lungs, liver, stomach, bladder, kidneys and small intestines. In a second experiment, a wound in a dog's neck was dressed every morning with tartar emetic ointment, the dog dying at the end of the seventh day. In this case no antimony was found in the brain, but it was found in larger quantities in the liver and spleen than in the other organs. It is very evident, therefore, that tartar emetic, and presumably other salts of antimony likewise, will penetrate all the tissues of the body and that at the same time the antimony is constantly being eliminated by the kidneys. Further than this, the few results recorded indicate nothing definite. As Dr. Richardson well says, "the election of antimony by different parts of the body is as yet an open question; that the liver, however, would appear to be the structure in which it is most collected when the administration is slow and in small doses; and that the elimination of the poison is attempted by all the secreting surfaces." No positive statements can therefore be made regarding the relative distribution of antimony, other than in a general way.

Hence, it has been our object in the present investigation to study somewhat in detail, the relative distribution of antimony in the different tissues of the body under varying conditions; both as to the form of the poison and the manner of its introduction. As with arsenic, so with antimony, the relative proportion of poison found in the different tissues after death may become of considerable medico-legal importance, provided we have sufficient confirmatory data from which to draw conclusions. Particularly is it of importance to know the way in which the form of the poison will influence its distribution. Whether as with arsenic,† the administration of a soluble and diffusible form of the poison will lead to a noticeable accumulation in the brain or nerve tissue in general.

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*See Woodman & Tidy, p 129. Also Dr. W. B. Richardson, Abstract in Amer. Jour. Med Sciences, 1857, vol. 33, p. 266, and B. and F. Med. Chirurg. Rev. Oct. 1856.

† See Amer. Chem. Jour., vol. v, p. 8, also Studies from the Laboratory of Physiological Chemistry, S.S.S. of Yale College, 1884–85, p. 141.

1. *The quantitative estimation of antimony.*

There are many methods by which antimony may be detected, even when present in quite small quantities; but there are only a few which yield accurate quantitative results, particularly in the presence of organic matter. In attempting to find a method sufficiently accurate for our purpose, we first tried the method recommended by Orfila, of introducing the final antimony solution into the Marsh apparatus; using for this purpose the form of apparatus and mode of procedure, found so efficacious in the case of arsenic.* In every trial, however, there was a loss of at least 40 per cent. of the antimony; thus in the first case, with an amount of tartar emetic equivalent to 6 milligrams of metallic antimony, only 3·4 milligrams of the metal were recovered, and under exactly such conditions as with arsenic would lead to the recovery of the entire amount. And since in this trial experiment, the antimony was introduced directly into the Marsh apparatus, as tartar emetic dissolved simply in dilute sulphuric acid and without the presence of any organic matter to act as a hindrance, it follows that the loss must be due to retention of a portion of the antimony by the zinc and platinum. A second trial, with the same amount of antimony salt, gave a decided deposit of metallic antimony in the heated tube, but weighing only 3·5 milligrams. In this case the apparatus ran for three hours, but as before, it is evident that only a portion of the antimony was converted into antimoniuretted hydrogen, the remainder undoubtedly being retained either by the platinum used to alloy the zinc, or by the zinc itself. The presence of a small amount of platinum fused in with the zinc, previous to its granulation, does not appear to offer any obstacles to the complete evolution of arsenic as arseniuretted hydrogen. Numerous results obtained by one of us† testify to the accuracy of this statement. Bernstein had also noticed that alloying zinc with platinum or silver, did not hinder the complete evolution of the arsenic, while the addition of a little platinic chloride solution to the acid fluid from which arsenic was being evolved, led to the precipitation of even 50 per cent. of the arsenic present.‡ This probably is the explanation of the low results obtained by Hedden and Sadler in the estimation of arsenic by the Marsh apparatus, in the presence of platinum.§ But with antimony,

* See Amer. Chem. Jour., vol. ii., p. 235. † See Amer. Chem. Jour., vol. ii, p. 235.
‡ See Dragendorff, Gerichtlich chemische Mittheilung von Giften, p. 334, foot note.
§ See Amer. Chem. Jour., vol. vii, p. 342.

either the platinum alloyed with the zinc is sufficient to retain fully 50 per cent. of the metal, or as is very probable, the zinc itself causes a precipitation of a portion of the antimony. That this is a point overlooked by most writers on chemical toxicology, is evident from a perusal of the literature on the subject.

Whether it would be possible to obtain all of the antimony as antimoniuretted hydrogen, in the absence of any metal other than perfectly pure zinc, we cannot say. Certainly as a method for quantitative purposes, it would be too tedious a process to admit of general use, especially where such small amounts of antimony, as in our own experiments, would limit galvanic action to a minimum. That the method is capable of showing the presence of very small amounts of antimony, is unquestionable. Wormley's experiments* are very decided on this point, but evidently only a portion of the antimony will be recovered.

Precipitation of the antimony by hydrogen sulphide and final oxidation by fuming nitric acid and weighing as Sb_2O_4, gave fairly satisfactory results, when the weight of antimony was not less than 10 milligrams of Sb_2O_4. With smaller amounts, the results were far too high, owing probably to the far greater proportional increase of sulphur. Attempts to weigh as sulphide, after fusion of the first hydrogen sulphide precipitate with potassium nitrate and carbonate, likewise gave too high results when the amount of antimony was small. Further, the two latter methods are somewhat unsatisfactory, in that when the amount of antimony is very small, the nature of the final products is such, it is difficult to be certain of the purity of the matter weighed.

Owing to this reason partly, we next turned our attention to the electrolytic method for the separation and determination of antimony, as in this case the appearance of the metallic mirror is in itself a fair guarantee of the nature of the deposit, and its purity is easily proved.

Alex. Classen† has shown the accuracy of the method in general quantitative work, where moderate amounts of antimony are present (0·15—0·2 grams Sb). In his experiments, the antimony, in the form of sulphide, was dissolved in ammonium sulphide and the solution then exposed to electrolytic action. The ammonium sulphide solution must be free both from polysulphides and from free ammonia.

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* Micro-Chemistry of Poisons, p. 229.

† Quantitative Analyse durch Elektrolyse. Berichte d. deutsch. chem. Gesell., xvii, p. 2474. See also Alex. Classen and Rob. Ludwig, ibid., xviii, p. 1104.

Classen also found that antimony was deposited quantitatively, when the sulphide was dissolved either in potassium or sodium monosulphide, or in potassium or sodium hydrosulphide. Polysulphides must also be absent in this case, and the potassium and sodium must be quite free from both iron and aluminum, as by long continued electrolytic action, sulphide of iron and aluminum hydroxide may be deposited upon the antimony. Classen also recommends the use of ammonium sulphydrate and a weak current; a strong current tending to cause the separation of the antimony in a pulverulent form, not closely adherent to the platinum.

As it would be necessary in our work, after oxidation of the organic matter, to separate the antimony as sulphide, the above method seemed particularly advantageous, and experiments were therefore tried to ascertain its value when applied to very small quantities. We employed a battery of four moderate sized gravity cells, giving a weak current, and as a rule, exposed the solution to the action of the current for at least 15 hours, as we found better results were obtained by the long continued action of a weak current, than by the quicker action of a more rapid one; particularly in such solutions as we usually had to work with, containing considerable excess of sulphur and some organic matter. The negative pole of the battery was either a small platinum crucible or a platinum dish, while the positive pole was a large piece of platinum foil welded onto a good sized platinum wire. The deposition of the antimony was much more complete, more tightly adherent to the platinum, and as a rule less mixed with sulphur under this arrangement, than when the dish was made to serve as the positive pole; due, doubtless, simply to the broader surface for the deposition of the metal.

Using ammonium sulphide as a solvent for the antimony sulphide, did not give us very good results by electrolysis, the loss being considerable. Much better results were obtained by using a solution of sodium monosulphide, made by saturating one-half of a 15 per cent. solution of sodium hydroxide with hydrogen sulphide, and then adding the remaining half of the sodium hydroxide solution.

The method was tested by precipitating definite volumes of a standard antimony (tartar emetic) solution with hydrogen sulphide, dissolving the metallic sulphide in the sodium monosulphide and then exposing the solutions to electrolytic action. When the separation of the metal was complete, it was found best to wash the deposit with considerable water, without breaking the current; as sometimes, as in the presence of tartaric acid, the separated metal rapidly dissolved

on discontinuing the current. The antimony was then finally washed with alcohol, any adherent sulphur lightly brushed off and the dish dried and weighed.

Following are some of the preliminary results obtained:

Standard Sb Solution.	Theoretical Am't Sb.	Am't Sb found.	Duration of Electrolysis.
50 c. c.	0·0375 gram.	0·0375 gram.	16 hours.
10	0·0075	0·0077	18
10	0·0075	0·0075	24
10	0·0075	0·0078	18
10	0·0075	0·0074	8
2	0·0015	0·0012	10
1	0·00075	0·0008	3

These results showed the method to be perfectly satisfactory for our purposes, and we, therefore, next tried the separation of small amounts of antimony from organic matter, and its final recovery by the above method.

As there was no doubt that large amounts of antimony could be satisfactorily recovered from organic matter, our experiments were confined mainly to very small quantities. In each experiment, 100 grams of either liver or beef were finely divided and to the so-prepared tissue, a few cubic centimetres of the standard antimony solution were added and the mixture *thoroughly* oxidized with hydrochloric acid and potassium chlorate. After removal of all free chlorine from the filtered fluid, by careful heating, the antimony was precipitated by hydrogen sulphide. This precipitate, which naturally contained, in addition to the sulphide of antimony, considerable sulphur and some organic matter, was then treated as follows: While still moist, after being freed from all hydrochloric acid by thorough washing, it was dissolved in the cold sodium monosulphide solution and then directly subjected to electrolysis. At first, we thought it necessary to free the precipitate from its excess of sulphur and organic matter by solution in ammonium sulphide, evaporation, fusion with potassium carbonate and nitrate, etc., obtaining it finally in the form of sulphide again, free from its former impurities. This, however, we found to be unnecessary; in fact the loss was far greater than the gain. Provided the oxidation with potassium chlorate be a thorough one and the free chlorine entirely removed from the solution, the first hydrogen sulphide precipitate is well adapted for electrolysis directly. It was found best, however, to keep the solution on the battery until all of the sulphur and organic matter was oxidized; that is until the reaction of the fluid had become acid. This took,

many times, 30 to 40 hours with our slow current. A more rapid current, would, to be sure, bring about a change in reaction much more quickly, but occasionally under such conditions the antimony would be less closely adherent and loss occur. Then again as the sulphur present was gradually changed into sulphuric acid, the final, strongly acid-reacting fluid, became a good conductor and so at the last, even with our four cells, electrolytic action was quite strong. In washing the deposited antimony, the acid fluid was syphoned out and water continuously added, without breaking the current, until the original fluid was entirely removed.

Following are a few of the results obtained, the antimony being added to 100 grams of tissue in each case.

Standard Sb Solution.	Theoretical Am't Sb.	Am't Sb found.	Duration of electrolysis.
10 c. c.	0·0075 gram.	0·0075 gram.	24 hours.
10	0·0075	0·0072	48
10	0·0075	0·0069	36
10	0·0075	0·0074	24
10	0·0075	0·0064	20
5	0·0037	0·0028	18

The results are certainly not all as close as those obtained in the absence of organic matter, but are perhaps as satisfactory as could be expected under the conditions of the experiment, viz: a large proportional amount of tissue (100 grams), a very small amount of metal and a large volume (say 500 c.c.) of fluid to precipitate from, with hydrogen sulphide. Even under the most unfavorable conditions, at least 75–80 per cent. of the antimony introduced into the organic matter was recovered.

We next turned our attention to the obtaining of some convenient and quick method for the direct determination of antimony in urine, or other like organic fluid. Preliminary experiments showed us that antimony, in the form of tartar emetic, could be separated completely from a tartaric acid solution by electrolysis. The separation takes place rapidly, but care must be taken to remove all of the tartaric acid solution by displacement with water, before breaking the current, otherwise the deposited antimony will instantly dissolve. Trial tests repeatedly gave results in close accord with theory. Excess of sodium tartrate, however, appeared to interfere somewhat with separation of the antimony; while the presence of sodium chloride in the presence of an excess of tartaric acid, prevented entirely the separation of the antimony. From a sulphuric acid solution, however, antimony was also deposited quantitatively, and on applying

this method to antimoniacal urine, we found it possible to recover the antimony without loss. The following results testify to the accuracy of the method:

In each experiment 25 c.c. of normal urine were employed, to which was added a number of cubic centimetres of the standard antimony solution and then 1 c.c. of pure dilute sulphuric acid, after which the solution was connected with the battery.

Standard Sb Solution in 25 c. c. urine.	Theoretical amount Sb.	Amount Sb found.	Duration of electrolysis.
10 c. c.	0·0075 gram.	0·0075 gram.	48 hours.
10 "	0·0075 "	0·0074 "	24 "
10 "	0·0075 "	0·0074 "	20 "
5 "	0·00375 "	0·0038 "	18 "
5 "	0·00375 "	0·0037 "	18 "
5 "	0·00375 "	0·0036 "	15 "
3 "	0·00225 "	0·0024 "	10 "
2 "	0·0015 "	0·0018 "	20 "
2 "	0·0015 "	0·0016 "	18 "
1 "	0·00075 "	0·0008 "	16 "

Doubtless all of these results could have been obtained equally as well in a very much shorter time, but most of the solutions were connected with the battery at night and allowed to run until morning, or whenever convenient. The method is evidently very accurate when applied in this manner, and we have made use of it very satisfactorily, even with 150 c. c. of urine, using in this case a platinum dish of 200 c. c. capacity as the negative electrode.

2. *Relative distribution of absorbed antimony.*

As already stated, the object sought in the following experiments was to ascertain the relative distribution of antimony under varying conditions; particularly, variations in the form of the poison, as its solubility or insolubility; in the method of introduction, as per mouth, rectum or sub-cutaneously; and lastly in the length of time during which the poison is being taken, whether in one large dose or in many small ones frequently repeated. All of these points we have endeavored to cover in the experiments about to be described.

Experiment I.

Hypodermic injection of a solution of tartar emetic.

0·120 gram of tartar emetic, dissolved in a little water, was introduced under the skin (right thigh) of a cat weighing 1262 grams.

3:10 p. m., solution injected.
3:16 " vomited copiously.
3:20 " " again, simply mucus.
3:23 " " mucus and bile.
3:38 " " and purged.
3:45 " partially paralyzed.
3:54 " vomited again.
4:30 " much prostrated.
5:10 " dead.

The various organs were then separated and the absorbed antimony determined, according to the method already indicated. Following are the results:

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	52·0	6·35	12·21
Brain	27·5	0·60	2·18
Heart and lungs	32·0	0·70	2·18
Kidneys	12·0	0·15	1·25
Stomach and intestines	74·0	0·80	1·08
Muscle from back	138·0	1·25	0·90
	335·5	9·85	

These figures show the greatest absorption by the liver; the brain stands next, while the muscle tissue appears to have absorbed but a relatively small amount of the antimony. In this connection it must be remembered that two hours only, intervened between the introduction of the poison and the death of the animal, hence it is evident that the brain tissue must have a decided tendency to hold absorbed antimony. The antimony, however, was introduced in the form of a readily soluble salt and under conditions directly favoring rapid and wide-spread distribution. That there is certainly some decided selective action, is evident from the fact that the muscle tissue, which must truly have had as good an opportunity as the brain tissue, retained per 100 grams of substance far less of the poison. Elimination had evidently commenced, for the kidneys contained a decided amount of antimony.

Experiment II.

Hypodermic injection of a solution of tartar emetic.

In this experiment, a smaller amount of tartar emetic was used than in the preceding; and further, the poison was introduced in three distinct doses, thus allowing longer time for absorption. In fact, the animal lived 22 hours after the first dose, hence the experi-

ment stands in striking contrast to No. 1, in which the animal lived but two hours; while the results, contrasted with the preceding, show plainly the influence of time on the distribution of the poison. Following are the results of the experiment on a rabbit weighing 1295 grams.

Mar. 31, at 5:20 p.m.,	injected	under the skin of leg,	0·012 grm.	tartar emetic.
April 1, " 8:45 a.m.,	"	"	0·035	"
April 1, "12:45 p.m.,	"	"	0·035	"
		Total,	0·082	

Animal died at 3:05 p. m.

Following is the distribution of the antimony:

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Kidneys	11·5	0·60	5·21
Liver	63·0	1·50	2·38
Brain	9·0	0·20	2·22
Stomach and intestines	98·0	2·00	2·04
Heart and lungs	17·0	0·25	1·47
Muscle from back	106·0	0·70	0·66
	304·5	5·25	

As might naturally be expected, the results indicate a more even distribution of the poison than in the preceding experiment. Although two-thirds as much antimony was used as in experiment No. 1, the liver contains a far smaller proportional amount of the poison than in the preceding experiment, while the kidneys stand first in their content of antimony. Between the brain and the liver, there is but little difference and the experiment plainly substantiates the preceding in showing the tendency of brain tissue, under these conditions, to absorb and retain antimony. In the muscle tissue the percentage of absorbed antimony is almost exactly the same as in No. 1, that is, proportional to the amount of antimony introduced. The animal had evidently lived long enough to admit of a fairly complete distribution of the poison, and elimination having been going on for some time, those parts which had originally contained the most, particularly the liver, had been drawn on to the greatest extent; so that at the time death intervened, the excretory organs, notably the kidneys, were the richest in poison. This fact further indicates that the elimination of absorbed antimony proceeds somewhat rapidly.

Experiment III.

Hypodermic injection of a solution of tartar emetic.

In this experiment, a cat weighing 1613 grams, had injected under the skin of its hind leg 0·150 gram of tartar emetic in one dose. There was some purging and vomiting, and the animal died in 4¼ hours after the administration of the poison. The object sought in this experiment, which is virtually a repetition of No. 1, with a somewhat larger dose of antimony, was simply to see whether there would be found the same relative absorption of antimony by the liver and kidney as in No. 1, and if by chance there should occur a longer interval of time between the introduction of the poison and death, what then would be the relative amounts of antimony in the two organs. As stated above, the animal lived 4¼ hours after the administration of the poison, or 2¼ longer than the cat in No. 1. Following are the results of the analysis of the two organs:

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	62·0	2·50	4·03
Kidneys	14·5	0·25	1·72

These confirm to a certain extent the results of No. 1, while at the same time the smaller difference between the amount of antimony contained in the liver and kidneys, as compared with the difference found at the end of two hours (see experiment I), would seem to indicate that the liver had already absorbed its maximum amount, and that at the time of death, elimination was well under way; or in other words, that the removal of the absorbed antimony from the liver had already commenced.

Experiment IV.

(*a.*) *Hypodermic injection of a solution of tartar emetic.*
(*b.*) *Injection of a solution of tartar emetic per rectum.*

These two experiments were undertaken to ascertain whether the avenue by which the poison was introduced, would influence materially the relative absorption of the antimony. The results, however, although interesting, do not definitely answer the question. Absorption by injection per rectum is so much slower than by hypodermic injection, or the effects produced are so much slower in manifesting themselves, that it is impossible to have the conditions exactly alike in the two cases. Either the time required to produce a given

effect, in the case of injection per rectum, will be longer than by hypodermic injection, or else the amount of poison must be correspondingly increased; either of which introduces an objectionable element into the experiment.

(*a.*) Rabbit weighing 1485 grams had injected under its skin 0·80 gram of tartar emetic dissolved in a little water. Injection made at 11:35 a. m. At 3:45 p. m., 4 hours and 10 minutes after the first injection, 0·08 gram more was injected in the same manner. At 4:05 p. m. the animal died.

(*b.*) Rabbit weighing 1512 grams had injected per rectum 0·08 gram of tartar emetic dissolved in a little water. Injection made at 11:45 a. m. At 3:50 p. m. the animal apparently not being affected at all, whereas rabbit (*a*) was strongly under the influence of the poison, 0·160 gram more of the salt was injected per rectum as before. At 5:30 p. m. the animal was still alive, but evidently feeling the effect of the poison. The animal died during the night.

Following are the results of the analysis of the parts from the two rabbits:

RABBIT (*a*) *hypodermic injection.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Kidneys	10·2	0·65	6·34
Brain	5·5	0·20	3·63
Liver	54·0	1·30	2·40
Heart and lungs	15·5	0·30	1·93
Stomach and intestines	174·0	1·60	0·92
Muscle	110·0	0·50	0·45
	369·2	4·55	

RABBIT (*b*) *injection per rectum.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Stomach and small intestines	172	8·89	15·30
Brain	9	0·40	4·40
Rectum and adjoining intestine	18	0·55	3·05
Liver	54	1·60	2·96
Kidneys	18	0·25	1·92
Muscle	100	1·10	1·11
Urine	20	0·20	1·10
Heart and lungs	17	*trace*	
	403	12·99	

Comparing first, the results obtained from rabbit (*a*) with those of the three preceding experiments, we see at once that the distribution of the antimony is much the same as in No. 2, in which, however, the animal lived nearly 22 hours after the introduction of the first dose of poison and somewhat over two hours after the last. The fatal dose, moreover, in this case was nearly the same in amount as the first dose in experiment IV *a*.

The conditions, however, of this experiment (IV *a*) do not exactly accord with any of the preceding, hence close comparisons cannot well be made. The brain, as in all of the experiments with tartar emetic, contains a proportionally large amount of antimony, while the muscle contains a very small amount. The only thing in this experiment not exactly in accord with the preceding experiments, is the proportionally large amount of poison in the kidneys, as compared with the liver. The only apparent explanation seems to be that, the first dose being small, the liver had quickly reached its maximum absorption and elimination had been rapidly going on; so that at the end of the four hours intervening between the first and second doses of the poison, the kidneys had drawn extensively from the liver, rapidly diminishing its content of the poison. Further, after the second dose of poison, the time before death was so short that the additional absorption by the liver was not sufficient to make up the deficiency, and hence the results found. In this connection, it must be remembered that tartar emetic is very readily soluble and diffusible, and that being injected in solution directly under the skin, its absorption must necessarily be very complete and rapid.

In Rabbit (*b*) the conditions are wholly different from those of the preceding experiments. In all, 0·24 gram of tartar emetic, dissolved in water was introduced into the rectum and 8–10 hours, at least, must have intervened between the administration of the first dose of the poison and death. That the stomach and small intestines should contain the largest proportional amount of antimony is perhaps not at all strange, since the antimony solution would naturally pass rapidly by osmosis through the entire alimentary tract. That this, however, is not the full explanation, is evident, when we compare the amount found in the large intestine with the former. If due simply to osmosis, the percentage amount of antimony would be about the same all through the intestines; hence we must look to some selective action for explanation of the increased amount found in the small intestines. In all of the preceding experiments, the amount of antimony found in the stomach and intestines has been

considerably greater than in the muscle tissue. Undoubtedly, the greater vascularity of the former has much to do with its greater content of the metal, but even this is not sufficient to account for all of the antimony found; for whenever blood itself has been analyzed, the amount of antimony has not been large. Unquestionably then, we must assume special absorptive action on the part of the epithelial cells of the stomach and small intestines. In this connection it is well to notice the work of Brinton, who proved that when tartar emetic was injected into the vein of an animal, it was very freely and rapidly eliminated by the stomach. This was also corroborated by Dr. Richardson who, in addition, found that a similar elimination followed the inhalation of antimoniuretted hydrogen.* In addition, it may be that absorption of antimony from the alimentary tract goes on slowly and that hence only a portion was removed. This idea has considerable to support it, when we consider the distribution of the absorbed antimony. Remembering that in this experiment, a larger amount of antimony was used than in any of the preceding ones, and that there was apparently ample time for absorption, one cannot help but think that the content of antimony in the remaining tissues and organs is very small. This is very evident, and must be due to one of two causes; either there has been a lack of absorption or else elimination has been going on very rapidly. The brain contains a noticeable amount of antimony, larger than found in any preceding case, while the liver and kidneys both contain a comparatively small amount. The amount of antimony in the kidneys and particularly the amount in the urine, plainly indicates that elimination was going on rapidly; but the fact that the percentage content of antimony in the liver is greater than in the kidneys, would perhaps indicate that at the time of death, absorption was not completed. Such being the case, the only inference to be drawn from the two preceding experiments, is that the introduction of tartar emetic into the rectum leads simply to a much slower absorption and distribution of the antimony than by hypodermic injection, but that there is no essential difference in the relative distribution of the poison under these two conditions.

* Quoted by H. C. Wood, Therapeutics, p. 159.

EXPERIMENT V.

(*a.*) *Tartar emetic in substance, introduced into the stomach.*

(*b.*) *Antimonious oxide* (Sb_2O_3) *introduced into the stomach.*

In this experiment there were two objects in view; one was to see the effect of tartar emetic in substance, as compared with the action of the same salt introduced into the system by hypodermic injection or per rectum; the second, to compare the absorption of an *insoluble* compound of antimony (Sb_2O_3) with that of the more soluble tartrate.

In this experiment two dogs were used and the poison was fed to them at regular intervals, in small doses, for a period, in each case, of 17 days. The animals were then killed and the various parts analyzed. The two experiments were exactly alike in every respect, except in the amount of poison administered.

(*a.*) Dog weighing 7·75 kilos was fed 0·762 gram of tartar emetic during a period of 17 days, in two or three doses daily, the individual doses being small enough not to induce vomiting. The first two days, the dose was 0·016 gram per day, the third 0·020 gram, the fourth 0·030 gram and so on, increasing each day until the last daily dose was 0·085 gram of the poison. The dog was then killed by chloroform, just six hours after the last dose of poison was administered.

(*b.*) Dog weighing 14·2 kilos was fed 2·073 grams of antimonious oxide, during a period of 17 days, in two daily doses of from 0·032 to 0·125 gram per day. The dog was then killed by chloroform, 18 hours after the last dose of antimony was given.

Following are the results of the analysis of the various parts:

DOG (*a*) *with tartar emetic*—(0·762 gram).

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	304	17·80	5·85
Salivary glands	11	0·25	2·27
Kidneys	58	1·25	2·15
Brain	76	1·15	1·51
Tongue	36	0·40	1·11
Muscle (thigh)	150	1·60	1·06
Spleen	19	0·15	0·80
Heart	77	0·50	0·66
Lung	140	0·50	0·36
Bone (femur and tibia)	43	0·10	0·23
Blood	130	0·20	0·15
Testes	12	*trace*	
Pancreas	23	*trace*	
	1079	24·05	

Dog (*b*) *with antimonious oxide*—(2·073 grams).

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	452	23·70	5·24
Lungs	140	1·80	1·28
Muscle (fore leg)	157	1·20	0·76
Brain	79	0·40	0·50
Muscle (thigh)	200	0·90	0·45
Kidneys	82	0·10	0·12
Heart	117	*trace*	
Blood	440	*trace*	
	1667	28·10	

In considering these results, we notice first that in dog (*a*) the distribution of the poison is much the same as in the preceding experiments with tartar emetic, viz: the liver, kidneys and brain stand first in their content of antimony. That the liver should contain more per 100 grams than the kidneys, although the animal lived full eight hours after the last dose of poison was taken, is here to be expected, since absorption as a whole would naturally be slower than in some of the preceding experiments; and, further, in this case probably all of the antimony would be absorbed through the portal circulation. In the case of dog (*b*), the conditions are different from any heretofore; we have here an insoluble form of antimony contrasted with a readily soluble and diffusible salt. Solution must necessarily be somewhat slow in this case, but the acid juices of the stomach unquestionably do dissolve and render diffusible, at least a portion of, this form of the poison.

We notice first that the total amount of antimony administered, is fully three times as much as the amount of tartar emetic given, and yet the amount of antimony recovered from the different tissues and organs is but 4 milligrams more than in the case of tartar emetic. This suggests that either considerable antimony is excreted by the kidneys (more than in the case of tartar emetic) or else that considerable passes through the alimentary tract unabsorbed. The dog being confined in a cage of suitable construction, the 24 hours' urine was collected on several occasions and the amount of antimony determined. Thus on one day, when 0·097 gram of antimonious oxide had been administered, following after a daily dose of 0·064 gram, the 24 hours' urine contained 13·5 milligrams of antimony (Sb). Later, at a time when the daily dose was 0·130 gram of the oxide, the 24 hours' urine contained 22·5 milligrams of antimony. Hence it is plain that

considerable of the antimony given was being absorbed; but bearing in mind that this latter amount was the largest excreted by the kidneys in any one day, and further that the daily dose of antimony was being increased each day rather than diminished, it is also plainly evident from the amount of absorbed antimony found, that a certain portion must pass through the alimentary canal unabsorbed. Further, the small amount of antimony found in the kidneys supplements this view, as does also the noticeably small amount of absorbed antimony found throughout the body, aside from the liver.

One of the main objects in trying this last experiment was to see what influence the form of the poison would have on its absorption by the brain. With arsenic, it has been plainly demonstrated by one of us,* as well as by other workers in this field, that soluble and readily diffusible forms of arsenic are absorbed by the brain in appreciable quantities, while arsenious oxide for example, no matter whether taken in large or small doses, single or oft-repeated, is never found in the brain other than in mere traces. With antimony we had expected to see something of the same kind. The results, however, although tending in that direction, are not quite as decisive as we should have liked. The antimony found in the brain in the antimonious oxide case is, to be sure, considerably smaller in amount than that found in (*a*), although the dose of antimony given in the former was much larger than in the latter case. But it is also to be seen in the antimonious oxide case, that the amount of absorbed antimony in the brain, although very small, is still greater than the amount found in the kidneys or muscle.

We attempted another experiment in the same direction with rabbits, but as the amounts of antimony found in the brain in both animals were hardly more than mere traces, the results do not give us any additional light on the matter. In spite of the fact that the experiment was a failure, so far as its main object was concerned, we venture to describe it, since it well illustrates in other respects, the greater virulence and diffusibility of tartar emetic. Two rabbits of nearly equal weight were selected, and to one potassium antimony tartrate was fed in gradually increasing doses for a period of 17 days, at the end of which time the animal died with all the symptoms of antimoniacal poisoning. To the other rabbit, antimonious oxide was fed for the same period of time, in doses the same as given to the first rabbit; that is, doses equivalent to the antimony (Sb) contained in the tartar emetic. Each rabbit, therefore, received

* See Studies from this Laboratory, vol. i, for the year 1884–85, p. 141.

twice a day, the same equivalent of antimony, and at the end of the 17 days the one rabbit had taken 2·34 grams of tartar emetic, the other 1·08 grams of antimonious oxide. While each rabbit had taken the same amount of antimony, the one which had taken it in the form of potassium antimony tartrate was much more severely affected by the poison. In this case there was severe purging and finally death on the 17th day. In the case of the rabbit fed with antimonious oxide, the only apparent effect of the poison was a loss of appetite and great thirst. This animal was killed with chloroform on the death of the first rabbit. Both forms of antimony were administered as powders, by way of the mouth, in small gelatin capsules.

Following are the results of the analysis of the various parts from the two rabbits:

RABBIT (*a*) *fed with tartar emetic.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	50·0	4·8	9·60
Kidneys	6·7	0·5	7·40
Heart and lungs	18·0	0·4	2·22
Muscle from back	55·0	0·5	0·91
Muscle from legs	79·0	0·3	0·38
Brain	7·7	*trace*	
	216·4	6·5	

RABBIT (*b*) *fed with antimonious oxide.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	57·0	1·3	2·28
Muscle from back	77·0	0·7	0·90
Muscle from legs	100·0	0·7	0·70
Kidneys	8·0	*trace*	
Heart and lungs	16·0	*trace*	
Brain	8·5	*trace*	
	266·5	2·7	

Looking at these results and remembering that each animal received the same amount of metallic antimony, it is evident that tartar emetic is much more completely absorbed than the oxide. With tartar emetic, however, the results are not exactly in accord with the previous ones, obtained with this salt; thus the amount of antimony absorbed by the brain is far smaller proportionally than

found hitherto. To be sure, the compound was not in the previous experiments introduced into the stomach of a rabbit in the form of powder, and it is possible that the reason for the difference in the amount of antimony found in the brain in this case and that in the brain of the dog similarly treated, lies in the fact of a slower absorption from the stomach of a herbivorous animal.

Influence of Antimonious Oxide on Metabolism.

By R. H. Chittenden and Joseph A. Blake.

The physiological action of antimony has been studied mainly with potassium antimony tartrate, the form in which antimony is most commonly used therapeutically. No experiments, however, appear to have been made, even with this salt, to ascertain the influence of antimony on the metabolism of the body. Gäthgens, however, as quoted by Dr. H. C. Wood,* found in some incomplete experiments an increase in the elimination of urea after repeated non-toxic doses of antimony. It is further reported† that antimonic acid or other preparations of the metal, when taken in half gram doses daily for about two weeks, cause a diminution in the amount of glycogen in the liver and even a total disappearance of it, and that the liver, kidneys and heart undergo fatty degeneration. Grohe and Mosler‡ have confirmed the latter and state that in the production of the famous fatty livers, a certain amount of the white oxide of antimony is fed to the geese daily. Aside from these facts, there appears little definite regarding the action of antimony on the physiology of nutrition.

What we have, therefore, endeavored to ascertain in the present experiment is the influence of antimony on metabolism; or particularly, on proteid metabolism as manifested in the excretion of nitrogen, sulphur and phosphorus. Previous experiments§ have shown that potassium antimony tartrate has a noticeable retarding action on pancreatic digestion; we have not, however, deemed it best in the present experiments to use tartar emetic, as the ready solubility and diffusibility of the compound might cause too rapid absorption and thus lead to speedy toxic action. In spite, therefore, of the fact that we have not made any experiments on the influence of antimonious oxide on digestive action, we have preferred to use the latter in the present experiments, because of its probable slower toxic action and also because it has been so extensively used as a means to induce, or to aid in the production of, fatty degeneration.

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* Therapeutics, Materia Medica and Toxicology, p. 156.

† See Virchow's Archiv., 1865, Band xxxiv, p. 78.

‡ Compare H. C. Wood. Therapeutics, p. 161.

§ Studies from this Laboratory, 1884–85, p. 105.

Our experiments were made on a setter dog, weighing 12·6 kilos. The animal was confined in a suitable cage, so that the excretions could be collected daily without loss. The food consisted of fresh beef and crackers, together with a suitable amount of water. The beef was prepared as follows: About 40 lbs. of fresh beef, freed from fat, tendons, etc., was finely divided by passing through a sausage machine and then dried at a low temperature until it had lost about 75 per cent. of water, and was in a condition suitable for preservation. 50 grams of this preserved meat, together with 75 grams of the sampled crackers, soaked in 300 c.c. of water, were fed to the dog twice daily. The meat, as determined by Kjeldahl's method, contained 12·4 per cent. of nitrogen, while the crackers contained 1·9 per cent. Hence the dog was fed daily 15·25 grams of nitrogen.

On May 11th, the dog was put upon this diet and from the 17th on, the 24 hours' urine was collected daily and analyzed. After a period of two weeks, during which daily analysis of the urine had shown a fairly constant composition, antimonious oxide was added to the diet in the quantities indicated in the table of results; the diet of course continuing the same throughout the length of the experiment.

We deemed it better, as well as more accurate, to measure the influence of the antimony by a daily determination of the total nitrogen, sulphur and phosphorus of the urine, rather than to attempt a determination of urea, uric acid, phosphoric acid, etc. Nitrogen, we determined, according to the method of Kjeldahl,* modified slightly as suggested by Dr. E. H. Jenkins, of the Agricultural Experiment Station, viz: 5 c.c. of the acid urine were placed in a long pear-shaped bulb and evaporated to dryness quickly on a water bath. The residue was heated directly over a small flame with 10 c.c. of pure concentrated sulphuric acid and 0·7 gram of oxide of mercury, until oxidation was almost complete. Then, a little finely powdered potassium permanganate was added, to render the oxidation quite complete. The solution was then diluted, an equivalent amount of potassium sulphide added to convert the mercury into sulphide, and lastly a standard solution of sodium hydroxide, after which the ammonia was driven off by boiling and collected in standard acid.

Total phosphorus and sulphur were determined as follows: 50 c.c. of urine were evaporated in a capacious silver dish with 10 grams of potassium hydroxide and 10 grams of potassium nitrate and the resi-

* Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Zeitschrift für analytische chemie, xxii, 366.

due heated carefully until the organic matter was completely oxidized. The fused mass was then dissolved in water and diluted to 250 c.c. Of this, 100 c.c., representing 20 c.c. of the original urine, were used for the sulphur, while the second 100 c.c. were used for the phosphorous, determination. For sulphur, the 100 c.c. were acidified with hydrochloric acid and evaporated to dryness on a water bath in order to remove all nitrate and nitrite. The residue was then dissolved in water acidified with hydrochloric acid, and the sulphuric acid precipitated with barium chloride in the usual manner. For phosphorus, the 100 c.c. were acidified with nitric acid, evaporated to dryness, the residue dissolved in water, acidified with nitric acid and the phosphoric acid precipitated with molybdenum solution. This precipitate was then dissolved in a dilute solution of ammonia, the phosphoric acid reprecipitated as ammonio-magnesium phosphate, and the phosphorus finally weighed as magnesium pyrophosphate. Chlorine was determined volumetrically in the usual manner, with a standard solution of silver nitrate, after destruction of the organic matter by fusion with potassium nitrate, etc.

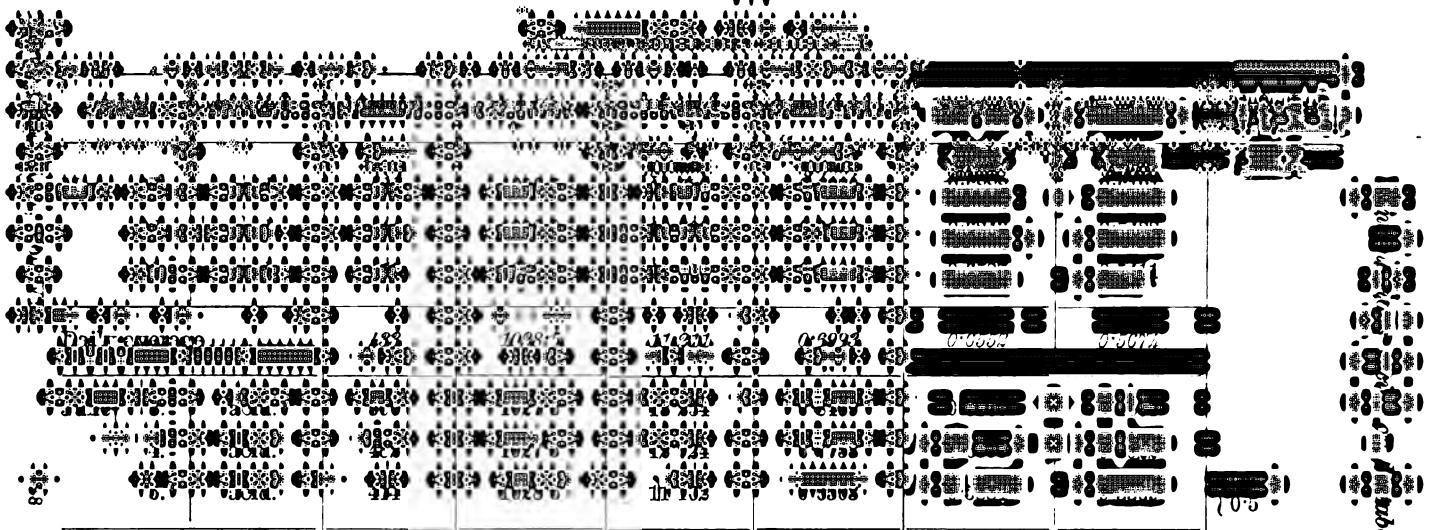
The results, expressed in grams per 24 hours, are shown in the accompanying tables. The 24 hours' urine represents the quantity passed from 9 A. M. of one day to 9 A. M. of the next. As, however, the animal was not always regular in its passage of urine, it frequently happened that the quantity on one day would be very small, while on the next it would be correspondingly increased, without any change in specific gravity, and with a daily average corresponding to the normal, as for example on May 25th and 26th.

In order, therefore, to obviate the difficulty which this irregularity tends to introduce into the results, we have added to the table a daily average of each three days results; a study of which shows plainly that antimonious oxide, in the present experiment at least, does not have any noticeable influence on the excretion of any of the elements determined. Numerically, there is a slight increase in the amount of nitrogen excreted during the taking of the antimony, but the increase is noticeable only in the grand average and is altogether too small to be of much significance. Further, it is to be noticed that the average for the two series does not show any corresponding increase in sulphur. If antimony causes an increased excretion of nitrogen, it means an increase in proteid metabolism, which should in turn give rise to an increased excretion of sulphur and phosphorus. It is to be noticed in the daily results, that the excretion of sulphur nd phosphorus runs parallel with the excretion of nitrogen; an in-

WITHOUT ANTIMONY.

Date.	Reaction.	Volume.	Sp. Gr.	Nitrogen.	Phosphorus.	Sulphur.	Chlorine.	Amount of Sb_2O_3 taken.
		c. c.		grams.	gram.	gram.	gram.	grains.
May 17.	acid.	462	1024·5	10·526	0·6122	0·5789	0.7239	
18.	acid.	510	1024·5	10·949	0·6587	0·6289	0·7203	
19.	acid.	469	1027·5	11·898	0·7855	0·6716	0·5871	
Daily average		*480*	*1025·5*	*11·124*	*0·6688*	*0·6264*	*0·6771*	
May 20.	alkaline.	470	1023·5	9·688	0·5716	0·5606	0·6720	
21.	acid.	466	1027·0	11·904	0·7118	0·6958	0·8792	
*28.	acid.	468	1029·5	13·226	0·7646	0·7738	0·5126	
Daily average		*468*	*1026·6*	*11·589*	*0·6826*	*0·6765*	*0·5212*	
May 24.	acid.	406	1030 0	11·492	0·6586	0·6654	0·4574	
25.	acid.	392	1027·0	9·512	0·5599	0·6193	0·7852	
26.	acid.	648	1027·5	16·294	1·2396	0·9709	0·9829	
Daily average		*482*	*1028·1*	*12·432*	*0·8177*	*0·7518*	*0·7251*	

* On the 22d a portion of the urine was lost.



Daily average.....	455	1027·8	12·360	0·7575	0·6445	0·3056
Average for the series...	466	1027·3	11·743	0·7251	0·6708	0·5592

† Between the 29th of May and the 3d of June the urine was not analyzed, as the dog seemed somewhat unwell and in poor appetite.

WITH ANTIMONIOUS OXIDE.

Date.	Reaction.	Volume.	Sp. Gr.	Nitrogen.	Phosphorus.	Sulphur.	Chlorine.	Amount of Sb_2O_3 taken.
		c. c.		grams.	gram.	gram.	gram.	grains.
June 6.	acid.	490	1030·0	12·968	0·9011	0·6743	0·6521	0·5
								0·5
7.	acid.	350	1030·0	10·060	0·5961	0·5018	0·4601	0·5
								0·5
8.	acid.	560	1026·5	13·226	0·8146	0·7780	0·6361	0·5
								0·5
Daily average.....		*466*	*1028·8*	*12·084*	*0·7706*	*0·6513*	*0·5827*	
June 9.	acid.	432	1030·0	12·587	0·7576	0·6333	0·4597	0·5
								1·0
10.	acid.	474	1027·5	11·838	0·7068	0·6275	0·6898	0·5
								0·5
11.	acid.	424	1028·0	11·890	0·7131	0·5977	0·3450	1·0
								0·5
Daily average.....		*443*	*1028·5*	*12·105*	*0·7258*	*0·6161*	*0·4981*	

WITH ANTIMONIOUS OXIDE—*continued.*

Date.	Reaction.	Volume.	Sp. Gr.	Nitrogen.	Phosphorus.	Sulphur.	Chlorine.	Amount of Sb_2O_3 taken.
		c. c.		grams.	gram.	gram.	gram.	grains.
June 12.	acid.	504	1030·0	13·648	0·8550	0·7212	0·3536	0·5 1·0
13.	acid.	416	1027·0	10·645	0·6320	0·5307	0·5972	0·5 0·5
14.	alkaline.	478	1028·5	12·900	0·7490	0·8381	0·3571	1·0 0·5
Daily average.....		*466*	*1028·5*	*12·397*	*0·7453*	*0·6966*	*0·4359*	
June 15.	acid.	404	1030·5	11·471	0·6802	0·6788	0·5563	1·0 1·0
16.	acid.	484	1028·0	12·173	0·7664	0·6414	0·5453	1·0
17.	acid.	432	1027·0	10·935	0·5797	0·6297	0·4326	1·0 1·0
Daily average.....		*440*	*1028·5*	*11·526*	*0·6754*	*0·6499*	*0·5114*	
Average for the series...		*453*	*1028·6*	*12·028*	*0·7292*	*0·6534*	*0·5070*	

crease in the latter is always accompanied by an increase in the two former. In the grand average of the results, however, the slight increase in nitrogen is not accompanied by a corresponding increase in sulphur. In fact, the two series of results, indicate plainly that the antimony was without any material action. The total amount of antimony given, 16 grains of the oxide during 13 days, was certainly sufficient in quantity to have exerted its peculiar influence if possessed of any. The antimony was certainly absorbed, and that too in considerable amount. Thus on the 11th of June the 24 hours' urine contained 13·5 milligrams of metallic antimony; on the 17th, 22·4 milligrams; on the 18th, 17·6 milligrams and on the 20th of June, 15·1 milligrams of metallic antimony. These quantities of absorbed antimony would certainly indicate the presence of sufficient antimony for some decided influence on metabolic action, if any existed. The amount of nitrogen excreted daily, is seen to be considerably below the amount of nitrogen ingested. We did not make daily examinations of the fæcal matter, but such as were made showed plainly that the deficiency in nitrogen was contained mainly in the fæces; thus on the 5th of June the 100 grams of fæces excreted, contained 2·42 grams of nitrogen. At that date, the average amount of nitrogen excreted by the urine was 12·36 grams per day; this amount, added to the fæcal nitrogen makes a total of 14·78 grams excreted, against 15·25 grams ingested; a difference to be found mainly in the hair thrown off, and in part, in the ordinary errors of analysis.

We must conclude, therefore, that small repeated doses of antimonious oxide are without influence on the excretion of nitrogen, sulphur and phosphorus, and that consequently this compound, at least when taken in non-toxic doses, has no action on proteid metabolism.

On Some Metallic Compounds of Albumin and Myosin. By R. H. Chittenden and Henry H. Whitehouse, Ph.B.

Ever since Lieberkühn, in 1852, attempted to establish the molecular weight of albumin by preparing and analyzing the copper compound resulting from the action of a soluble copper salt on a solution of egg-albumin, various investigations have been published bearing on the nature and composition of the compounds of albumin with the heavy metals. Before this time even, F. Rose, in 1833, had published an analysis of a copper compound of albumin in which he had found from 1·50 to 1·70 per cent. of cupric oxide, and Mitscherlich, in 1837, published an analysis of a similar albumin compound, in which he found from 2·8 to 3·3 per cent. of cupric oxide, while Lieberkühn's compound contained 4·6 per cent. CuO. Compounds of albumin with other metals have also from time to time been prepared, such as zinc, lead, silver and mercury, and in one or two cases provisional formulæ have been given. The results, however, are to be considered as quite uncertain. With platinum chloride a compound appears to have been formed* of somewhat more certain composition. Aside from the more recent experiments of Ritthausen† on the vegetable albumins (gluten-casein, legumin, etc.), egg-albumin has been the chief albuminous body experimented with, and copper the main metal.

Recent work by one of us (C) on the albumose and globulose bodies, together with work on the products formed from casein and myosin, has led to a partial study of the metallic compounds of these bodies. As a preliminary, however, we found it necessary to study a few of the compounds of egg-albumin, and as the results thus obtained were not in accord with the more recent results of Harnack‡ we have continued our work with egg-albumin and with myosin, the results of which we now present.

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* See Commaile, Moniteur Scientifique, 1866, and Fuchs, in Annalen der Chemie, vol. cli, p. 372.

† Die Eiweisskörper der Getreidearten, etc. Journal für prakt. Chem., vol. xii, p. 361.

‡ Untersuchungen über die Kupferverbindungen des Albumins. Zeitschrift für physiologische Chemie, vol. v, p. 198.

I. Egg-albumin.

(a) *Copper Compounds.*

In looking over the literature of the subject, it becomes evident at once that the older investigators, owing either to the nature of the compound, to adherent impurities or to faulty methods, were not able to obtain concordant results, since the copper compound of egg-albumin, as prepared and analyzed by six distinct investigators, was found to contain from 1·50 to 5·19 per cent. of CuO. In all of these cases the preparation of the copper compound was essentially the same; a solution of egg-albumin was precipitated with a solution of a copper salt, the precipitate collected, washed thoroughly with water, dried, and the copper determined by simple ignition. Naturally this method, as suggested by Harnack, might be expected to give too high results, since the copper precipitate would unquestionably retain considerable of the inorganic matter of the albumin. Treated in this manner, however, F. Rose,* as already stated, found the copper compound to contain from 1·50 to 1·69 per cent. of CuO. Mitscherlich,† who held that the copper precipitate was a compound of egg-albumin with the copper salt, found in his preparations 2·8–3·3 per cent. CuO, while Bielitzki,‡ who demonstrated that the precipitate was an actual compound of albumin with cupric oxide, found in his preparations 4·75–5·20 per cent. of CuO. Lassaigne, as quoted by Harnack, found 4·95 per cent. of CuO, Mulder§ 4·44 per cent., while Lieberkühn's‖ preparation contained 4·6 per cent. of CuO. Further, Ritthausen's copper compounds of the vegetable albumins were found to contain from 11·5 to 17·0 per cent. of CuO. These results collectively, would therefore seem to indicate that when egg-albumin is precipitated by a soluble copper salt, the resulting compound does not contain a definite proportion of albumin and cupric oxide, or else that there are a large number of albumin-copper compounds. More recently, however, E. Harnack,¶ from analysis of fifteen separate preparations, comes to the conclusion that there are two distinct compounds of albumin with copper; one containing 1·35 per cent. of Cu, the other 2·64 per cent. of Cu, indicating as Harnack suggests, a copper albuminate in the first case of the formula $C_{204}H_{320}N_{52}O_{66}S_2Cu$, in which Cu replaces two atoms of hydrogen in the albumin molecule, and in the second case, an albuminate of the formula $C_{204}H_{316}N_{52}O_{66}S_2Cu_2$, in

* Poggendorff's Annalen, vol. xxviii, 1833.
† Müller's Archiv. for 1837, p. 91.
‡ Dissertation, Dorpat, 1853.
§ Physiologische Chemie, 1844–51.
‖ Poggendorff's Annalen, vol. lxxxvi, 1852.
¶ Loc. cit.

which two atoms of Cu replace four of hydrogen. The results obtained by this investigator would certainly seem to warrant this conclusion, for the analytical data of the different preparations show but slight variations; 1·34–1·37 per cent. in the one case, and 2·48–2·74 per cent. in the other. Further, Harnack worked with nearly ash-free preparations, the compounds after their first precipitation and washing being dissolved in sodium carbonate and reprecipitated by careful addition of acid. By repeating this process several times, the ash of the preparation was almost entirely removed, while the relative proportion of copper and albumin was not affected. As to the conditions which determine the formation of one or the other compound, there seems to be little definite other than that in general, the compound with smaller content of copper was obtained when the precipitation took place in the presence of a slight excess of albumin, and the compound with larger content of copper when in the presence of an excess of the copper salt. In no case were the copper salt and albumin solutions mixed in definite proportions, yet in every case one of the two compounds was formed; further, Harnack states that when an amount of copper salt exactly sufficient to form the albuminate is added to a given quantity of albumin, no precipitate results; in other words an excess of the copper salt is necessary to insure a separation of the compound.

Harnack's results, therefore, differ from those of the preceding investigators in that definite compounds appear to have been formed in every case, and further, in that the compounds contain a lower percentage of copper than found by any other investigators aside from F. Rose. This latter, it will be remembered, found 1·50–1·69 per cent. of CuO; 1·69 per cent. being equal to 1·34 per cent. of Cu, one of the percentages found by Harnack. Harnack further states that the average of the analyses made by other investigators, aside from Rose, show about 4·4 per cent. of CuO, and assuming that the various preparations contained an amount of ash equivalent to about 1 per cent. (which amount Harnack found in his preparations before purification) the percentage amount of cupric oxide would be reduced to about 3·4 $=2·7$ per cent. Cu, or the amount found by Harnack in his highest copper compound. But as Rose's preparation was made by the simple addition of an aqueous solution of egg-albumin to the copper salt and the copper determined as oxide by simple ignition, it would seem necessary to make the same deduction of 1 per cent. also in this case, which would make Rose's compound contain far less CuO than found

by Harnack. Further, Rose* found that the serum of ox blood yielded a similar compound with cupric sulphate, which contained only 1·14 per cent. of CuO or 0·88 per cent. of Cu. Hence there would seem to be little in these earlier investigations to substantiate the results obtained by Harnack. Mörner,† however, working with alkali-albuminate, found that on precipitating a solution of alkali-albuminate with cupric sulphate, in the presence of an excess of alkali, he obtained a copper albuminate containing a percentage of cupric oxide corresponding closely with that found by Lieberkühn. When, on the other hand, he precipitated a nearly neutral solution of alkali-albuminate with cupric sulphate, then the percentage of copper in the copper albuminate amounted to only one-third that found by Lieberkühn, or an amount about equivalent to that found by Harnack in his lowest copper compounds. Mörner further found that by precipitating a calcium albuminate solution with cupric chloride, the albuminate combined on an average with 2·33 per cent. of CuO, or just one-half the amount required by Lieberkühn's formula, and considerably less than the amount contained in Harnack's largest copper compounds.

Preparation of the albumin solution.—In our experiments it was thought best, as far as possible, to avoid exposing the albuminate to the action of alkalies, hence especial care was taken to prepare the egg-albumin as free from salts as possible, so that it would not be necessary to purify the albuminate by reprecipitation. The whites of a large number of eggs were finely divided by scissors and by shaking with glass, then mixed with an equal volume of water and thoroughly shaken with air, after which the solution was strained through cloth. Globulin was then precipitated by the addition of dilute acetic acid (the acid added as long as a precipitate formed), the solution finally filtered through paper, after which the filtrate was made exactly neutral with sodium carbonate and again filtered. The fluid so obtained was then dialyzed in running water for many days, a little thymol being added to prevent putrifaction. The fluid finally obtained was perfectly neutral, clear and contained but a small amount of inorganic salts.

In forming the albuminate we employed both cupric acetate and cupric sulphate, using in each case the same volume of albumin solution, but varying the amount of copper salt. The copper salt was generally added as long as a precipitate formed. The albumin-

* Loc. cit., p. 139.

† Jahresbericht für Thierchemie, 1877, p. 8.

ate was washed on a pump with water, until no reaction could be obtained with potassium ferrocyanide for copper or with acetic acid and potassium ferrocyanide for albumin. The preparations were dried at 100° C., then powdered and further dried at 110° C., until of constant weight. The percentage of copper was first determined by simple ignition and weighing as cupric oxide. The oxide was then dissolved in dilute nitric acid, the copper precipitated as sulphide with hydrogen sulphide and weighed as subsulphide by ignition in hydrogen gas with a little sulphur. Each series was made from a distinct preparation of albumin and nearly every compound made, was analyzed in duplicate.

Following are the analytical results:

SERIES I.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·5621 gram.	0·0081 gram.	1·13	0·0070 gram.	0·97
b	0·5176	0·0078	1·19	0·0065	0·98

With $Cu(C_2H_3O_2)_2$.

2*a*	0·5479	0·0088	1·27	....	....
b	0·5703	0·0091	1·26	0·0078	1·08

SERIES II.

With $CuSO_4$.

1*a*	0·5278 gram.	0.0067 gram.	1·00	0·0053 gram.	0·79
b	0·7075	0·0083	0·93	0·0068	0·76

With $Cu(C_2H_3O_2)_2$.

2*a*	0·5697	0·0081	1·12	0·0071	0·98
b	0·5005	0·0073	1·15	0·0061	0·95

SERIES III.

With $CuSO_4$.

1*a*	0·6235 gram.	0·0085 gram.	1·07	....	....
b	0·6705	0·0091	1·07	0·0088 gram.	1.04

With $Cu(C_2H_3O_2)_2$.

2*a*	0·8302	0·0130	1·24	....	....
b	0·9400	0·0151	1·27	....	....

Series IV.

With $CuSO_4$.

No.	Amt. sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·3621 gram.	0·0047 gram.	1·02	0·0038 gram.	0·82
b	0·3478	0·0046	1·03	0·0039	0·89
2*a*	0·4318	0·0060	1·08	0·0040	0·71
b	0·3288	0·0047	1·12	0·0033	0·78
3*a*	0·4083	0·0060	1·15	0·0047	0·90
b	0·4332	0·0064	1·17	0·0053	0·96
4*a*	0·3374	0·0047	1·09	0·0042	0·97
b	0·4289	0·0062	1·14	0·0050	0·90

With $Cu(C_2H_3O_2)_2$.

5*a*	0·6358	0·0090	1·11	0·0073	0·91
b	0·5147	0·0071	1·08	0·0064	0·99
6*a*	0·5321	0·0075	1·12	0·0065	0·95
b	0·5125	0·0073	1·13	----	----
7*a*	0·7260	0·0125	1·36	----	----
b	0·6916	0·0120	1·37	0·0100	1·15

Series V.

With $CuSO_4$.

1*a*	0·4838 gram.	0·0083 gram.	1·36	0·0074 gram.	1·21
b	0·5083	0·0091	1·41	----	----

With $Cu(C_2H_3O_2)_2$.

2*a*	0·5044	0·0085	1·32	----	----
b	0·5042	0·0084	1·32	----	----

From the analyses of these 15 preparations it is to be seen that the percentage amount of metallic copper, determined as oxide by simple ignition, amounts on an average to 1·17 per cent. When, however, the copper is determined as subsulphide, by precipitation with hydrogen sulphide, and thus obtained free from ash, the percentage amount of copper falls on an average to 0·94 per cent. Cu. The preparations thus contain 0·23 per cent. of ash. We were not able to obtain any copper compounds with a much smaller content of ash than this, except by the use of methods which appear to affect the composition of the compound.

Harnack states* that he was able to obtain the copper albuminate quite free from ash by dissolving the freshly precipitated albuminate, after it had been thoroughly washed, in sodium carbonate, filtering and reprecipitating the compound by careful addition of acid. By repeating this process several times the adhering inorganic matter was entirely removed. It seemed questionable, however, whether this treatment might not induce some alteration in the compound.

The two following series of experiments were tried with the intention of throwing some light upon this point.

SERIES VI.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·6320 gram.	0·0091 gram.	1·15	0·0080 gram.	0·99
b	0·6980	0·0099	1·13	0·0086	0·98
2*a*	0·5428	0·0077	1·12	0·0070	1·01
b	0·5121	0·0077	1·19	0·0061	0·95
3	0·3146	0·0080	2·03	0·0068	1·71
		With $Cu(C_2H_3O_2)_2$.			
4*a*	0·6105	0·0085	1·11	0·0075	0·96
b	0·5836	0·0078	1·06	0·0072	0·99
5*a*	0·5996	0·0087	1·15	0·0081	1·06
b	0·5592	0·0082	1·16	0·0069	0·99
6	0·4139	0·0131	2·51	0·0115	2.19

In this series of experiments all of the preparations, as before, were made from the purified albumin. Nos. 1, 2, 4 and 5 were, after precipitation, simply washed with water until the washings gave no reaction either for copper or albumin. No. 3, after being washed in a similar manner, was dissolved in very dilute sodium carbonate and reprecipitated by neutralization with dilute hydrochloric acid. No. 6 was dissolved up twice in this manner and both preparations were finally washed free from chlorine. A glance at the analyses shows plainly that this treatment has tended to increase the percentage amount of copper in the albuminate; due, doubtless, either to withdrawal of a portion of the albumin by the sodium carbonate, or else to a partial dissociation of the compound by the long continued washing with water.

The first reprecipitation has apparently increased the amount of copper in the compound fully 0·7 per cent., the second reprecipitation 0·5 per cent. more.

* Loc. cit., p. 202.

Series VII.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·6326 gram.	0·0091 gram.	1·13	----	----
b	0·6156	0·0086	1·10	----	----
2*a*	0·4054	0·0081	1·60	0·0063 gram.	1·28
b	0·4995	0·0099	1·58	0·0074	1·19
3*a*	0·3870	0·0068	1·39	0·0049	1·02
b	0·3702	0·0066	1·40	----	----

With $Cu(C_2H_3O_2)_2$.

4*a*	0·6175	0·0085	1·08	0·0074	0·95
b	0·6069	0·0082	1·07	0·0077	1·00
5*a*	0·3282	0·0072	1·73	0·0055	1·34
b	0·3755	0·0083	1·75	----	----
6*a*	0·7503	0·0132	1·39	0·0104	1·10
b	0·7016	0·0121	1·38	----	----

This series was prepared in the same manner as the preceding. Nos. 1, 3, 4 and 6 were simply washed with water, while No. 2 was reprecipitated once and No. 5 twice, and both ultimately washed free from all soluble matters. The results show here the same increased percentage of copper, although not so marked as in the preceding series, when the albuminate is dissolved in sodium carbonate and reprecipitated. Further, the percentage of ash is not, as a rule, materially changed by this process; thus in No. 2, where the albuminate was reprecipitated once, the difference in the percentage of copper as determined by simple ignition and by precipitation as sulphide, amounts to 0·37 per cent., while in No. 3*a*, where the compound was not reprecipited at all, the difference is exactly the same.

In precipitating the albuminate, there is formed in the fluid a small amount of either sulphuric or acetic acid. Harnack, to avoid this, states that it is better, after adding the necessary amount of cupric sulphate to the albumin solution, to exactly neutralize the mixture with sodium carbonate. If, however, the greatest care is not exercised and excess of cupric sulphate avoided, even partial neutralization of the fluid will result in the precipitation of a portion of the copper and thus show an apparent increase in the copper of the albuminate. So far, however, as our results show, the small amount of sulphuric acid liberated in the formation of the albuminate does not affect the character of the compound. In the following series, after each precipitation,

the mixture was made as near neutral as possible with dilute sodium carbonate and the compounds then filtered and washed thoroughly with water. The copper in this series was determined simply by ignition.

SERIES VIII.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.
1*a*	0·4273 gram.	0.0068 gram.	1·26
b	0·3867	0·0063	1·29
2*a*	0·3167	0·0049	1·23
b	0·3535	0·0052	1·18
3*a*	0·2418	0·0030	1·00
b	0·3079	0·0039	1·00
4*a*	0·6210	0·0138	1·78
b	0·7091	0·0158	1·78

The results plainly show no appreciable difference in the composition of the albuminate under this change in the conditions, unless in No. 4 where a larger amount of copper is found than usual. It is our opinion, however, that the small amount of acid liberated by the reaction is not sufficient to cause any especial change in the character of the albuminate; neither, probably, does very dilute sodium carbonate in itself change the substance to such an extent that on neutralization it is not precipitated in nearly its original form, or at least that the action in this case is not any greater than that produced by water alone. In fact we are much inclined to the view that the long continued action of water will gradually but surely affect the composition of the albuminate, and that doubtless the change in the composition of the compound noticed in our experiments on solution of the substance in sodium carbonate and reprecipitation is due to the combined action of the alkaline fluid and of water. Harnack states that week-long treatment of the freshly precipitated albuminate with water will gradually cause dissociation of the compound, but that it can be easily and thoroughly washed without any decomposition whatever.

Our experience, however, leads us to question the correctness of this view. Ordinarily, it has taken us an entire day to completely wash the freshly precipitated albuminate, so that the wash-water should give no reaction whatever for copper or albumin. In precipitating the albumin solution with cupric sulphate, the albumin never appears to be completely precipitated and at the same time, as Harnack has observed, it is necessary to add more than the proportional

amount of copper salt to obtain any separation of the albuminate. As a result, the filtrate contains considerable albumin and copper, but even after several hours washing on the pump (the filtration is slow at the best) the precipitate still gives up traces of albumin, as shown by acetic acid and potassium ferrocyanide, long after all traces of copper have disappeared. It is not impossible to wash the compound and reach a point where the wash-water contains neither copper nor albumin, but when the washing goes on slowly and the water remains more or less in contact with the albuminate for 24 hours, then frequently the washings will show traces of albumin continuously, without our being able to reach a point where the test fails to give any reaction whatever, or to show any special change in the intensity of the reaction.

The following series of experiments would appear to substantiate this view. The first six were washed for about twelve hours, when no copper reaction could be obtained in the washings and only the slightest reaction for albumin. The last six were washed for sixteen hours, and finally stood over night on wet filters with more or less water on them. At the end of this time, the washings continued to show a reaction for albumin with acetic acid and potassium ferrocyanide, and indeed the reaction appeared to increase rather than diminish in intensity on further washing. The washings contained no copper.

Following are the results of the analyses:

SERIES IX.— *With* $CuSO_4$.

No.	Amt. sub. taken.	Wt. CuO.	Per cent. Cu.
1	0·4207 gram.	0·0052 gram.	0·99
2	0·4676	0·0058	0·98
3	0·4881	0·0062	1·00
4	0·3162	0·0037	0·91
5	0·2882	0·0032	0·89
6	0·1857	0·0024	1·02
7	0·2892	0·0050	1·34
8	0·4137	0·0060	1.13
9	0·4077	0·0060	1.18

With $Cu(C_2H_3O_2)_2$.

10	0·6067	0·0126	1·64
11	0·6889	0·0111	1·27
12	0·7126	0·0109	1·22

While the difference is not very great, it is a constant difference, and it is to be remembered that the last six compounds differ in no respects whatever from the first six, except in being subjected to the longer action of water.

In comparing now these different results, it is seen that we have not been able to obtain a copper albuminate with a higher content of Cu than 2·19 per cent., and this only as a result of two reprecipitations; a condition, which, from our experience, tends to alter materially the composition of the original precipitate. The average of the results obtained by simple precipitation, show a content of 0·94 per cent. of Cu. A study of the individual results, however, shows too great a variation to believe wholly in the existence of a single, stable copper albuminate. Either there are one or two definite compounds, which, being more or less unstable, are prone to change under varying conditions and thus give rise to the variations in the content of copper noticed, or else there are a number of definite compounds liable to be formed as the conditions are varied, all of which, however, must be more or less unstable. Glancing over the individual results, it is plain that an amount of Cu approximating to 0·96 per cent. is found altogether too frequently to be the result of chance. Doubtless this figure represents most closely the content of copper in the ordinary copper albuminate obtained by simple precipitation, while the majority of the variations from this figure are due mainly to dissociation.

Taking Lieberkühn's formula of albumin, the following copper albuminates would be possible:

$(C_{72}H_{112}N_{18}SO_{22})_3 + Cu - H_2 = 1{\cdot}29$ per cent. Cu.
$(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2 = 0{\cdot}96$ " " "
$(C_{72}H_{112}N_{18}SO_{22})_6 + Cu - H_2 = 0{\cdot}77$ " " "

For the first, in which Lieberkühn's formula for albumin is trebled, the percentage of copper corresponds nearly to the lowest results obtained by Harnack, while in the second formula the percentage of copper accords closely with the average of our results. Whether the weight of the albumin molecule is represented more nearly by the second formula than by the first we have not sufficient data to determine, but certainly our results with the copper albuminate show a lower percentage of copper than would correspond with the first formula. Further, it would appear that the copper albuminates are readily prone to change under slight provocation and that this point, in part, undoubtedly explains the reason for the great variation in the results obtained by so many workers.

(b) *Lead Compounds.*

Lieberkühn* states that the lead salt of albumin cannot be obtained pure; that the insoluable precipitate formed by the addition of either lead nitrate or basic lead acetate to a solution of albumin, is simply a mixture. With basic lead acetate, Lieberkühn obtained a precipitate containing 17·86 per cent. of lead oxide, while the precipitate formed with lead nitrate contained 12·78 per cent. of lead oxide.

With protein, Mulder† obtained precipitates on the addition of neutral lead acetate and lead nitrate, which contained respectively 12·45 and 12.68 per cent. of lead oxide, while basic lead acetate gave a precipitate containing 30·63 per cent. of lead oxide. Berzelius‡ states that neutral lead acetate precipitates both albumin and blood serum, but that the greater portion of the albumin remains dissolved in the fluid united with acetic acid. Basic lead acetate on the other hand precipitates the albumin completely.

These last statements accord with our own results; with a neutral lead salt only a small precipitate was obtained, the compound being soluble apparently in both excess of the lead salt and of albumin, while with basic lead acetate the albumin seemed completely precipitated.

Further, Berzelius‖ states, on the authority of Mulder, that if a solution of potassium albuminate be made as neutral as possible with acetic acid and then precipitated with lead nitrate, the lead albuminate so obtained contains on thorough drying 5·84 per cent. of lead oxide.

Following are some of the results of our analyses. The compounds were made from thoroughly dialyzed albumin and were washed free from both lead and any excess of albumin. The preparations were dried at 110° C. until of constant weight and the lead was determined first by simple ignition, with addition of a little ammonium nitrate. The lead oxide, after being weighed, was then dissolved in dilute nitric acid, the solution evaporated to a small volume, the lead precipitated with a little sulphuric acid, two volumes of alcohol added, and the lead sulphate finally filtered and washed with 95 per cent. alcohol. The sulphate was then ignited with proper precautions and from the weight obtained, the percentage of lead again calculated.

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* Poggendorff's Annalen, Band lxxxvi, p. 124.

† Lehrbuch der Chemie, Berzelius, ix, p. 29. ‡ Lehrbuch der Chemie, ix, p. 43.

‖ Lehrbuch, ix, p. 49.

SERIES I.

With neutral lead acetate.

No.	Amt. Sub. taken.	Wt. PbO.	Per cent. Pb.	Wt. $PbSO_4$.	Per cent. Pb.
1*a*	0·4972 gram.	0·0188 gram.	3·49	0·0209 gram.	2·85
b	0·3992	0·0150	3·48	0·0166	2·83

SERIES II.

With neutral lead acetate.

1*a*	0·5341	0·0183	3·16	0·0216	2·75
b	0·5381	0·0198	3·40	0·0222	2·80

With basic lead acetate.

2*a*	0·5832	0·0460	7·30	0·0580	6·77
b	0·5008	0·0391	7·22	0·0487	6·62

SERIES III.

With neutral lead acetate.

1*a*	0·8057	0·0262	3·01	0·0274	2·32
b	0·6522	0·0211	2·98	0·0226	2·36

With basic lead acetate.

2*a*	0·7290	0·0593	7·55	0·0615	5·74
b	0·7630	0·0631	7·66	0·0609	5·45

SERIES IV.

With neutral lead acetate.

1*a*	0·4121	0·0152	3·42	0·0136	2·25
b	0·4823	0·0176	3·37	0·0178	2·50

With basic lead acetate.

2*a*	0·7234	0·0651	8·34	------	----
b	0·5365	0·0482	8·33	------	----

SERIES V.

With a large excess of basic lead acetate.

1*a*	0·6822	0·2119	28·81	------	----
b	0·5429	0·1714	29·28	------	----
2*a*	0·5836	0·1923	30·56	------	----
b	0·5913	0·1960	30·76	------	----
3*a*	0·5478	0·1896	32·11	------	----
b	0·5427	0·1873	32·02	------	----

These results plainly indicate that more than one compound of lead is formed, especially so with basic lead acetate, the composition being dependent in this case on the amount of lead salt added. With neutral lead acetate, the variations in composition are not so marked and as it is hardly possible to prepare a lead albuminate free from salts, or to eliminate them wholly in the calculations, it is questionable how far the results should be trusted, except in a general way.

The formula $(C_{72}H_{112}N_{18}SO_{22})_4+Pb-H_2$ would require 3·10 per cent. Pb, while $(C_{72}H_{112}N_{18}SO_{22})_5+Pb-H_2$ would require 2·50 per cent. Pb. In the case of the albuminate formed with basic lead acetate, it is to be noticed that the compound made by the addition of a large excess of the lead salt, contains about five times as much lead as the ordinary basic lead compounds.

(c) *Iron Compounds.*

F. Rose* has made iron albuminate, both from egg-albumin and from the serum of ox-blood, by the simple addition of ferric chloride to the albumin solution. Two preparations made from egg-albumin yielded respectively 2·79 and 2·88 per cent. of ferric oxide. Rose found the albuminate, when freshly precipitated, easily soluble both in excess of ferric chloride and in excess of the albumin solution.

Our preparations were made wholly from dialyzed albumin, and when so prepared and thoroughly washed the compound was found almost wholly free from adhering salts, so much so that after a few trials we deemed it unnecessary to make the determinations of iron other than by simple ignition and weighing as ferric oxide.

Following are some of our results:

SERIES I.

With Fe_2Cl_6.

No.	Amt. sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·7033 gram.	0 0094 gram.	1·33	0·92
b	0·6430	0·0086	1·33	0·93

SERIES II.

1*a*	0·4683	0·0052	1·11	0·76
b	0·3854	0·0042	1·08	0·75

* Poggendorff's Annalen, xxviii, p. 140, 1833.

SERIES III.

No.	Amt. sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·4710	0·0071	1·50	1·04
b	0·5329	0·0083	1·55	1·08
2*a*	0·7387	0·0100	1·36	0·94
b	0·6355	0·0087	1·36	0·94

SERIES IV.

1*a*	0·4862	0·0063	1·30	0·90
b	0·5558	0·0073	1·31	0·91
2*a*	0·5115	0·0070	1·36	0·95
b	0·4954	0·0066	1·33	0·92
3*a*	0·4505	0·0063	1·39	0·97
b	0·4610	0·0066	1·43	0·99
4*a*	0·4817	0·0071	1·47	1·01
b	0·4571	0·0065	1·44	0·98
5*a*	0·4819	0·0065	1·34	0·93
b	0·3846	0·0051	1·33	0·93
6*a*	0·4086	0·0059	1·45	1·00
b	0·3392	0·0048	1·41	0·97
7*a*	0·3814	0·0050	1·32	0·91
b	0·4107	0·0053	1·29	0·90
8*a*	0·4452	0·0058	1·30	0·91
b	0·4833	0·0065	1·34	0·92

These results show a fairly close agreement with one single exception, in which case the percentage of iron is nearly 0·25 below the average. The average percentage, moreover, of ferric oxide is just about one-half that found by Rose. Further, the average percentage of iron (Fe) corresponds very closely with the average percentage of Cu in the copper albuminate. Eliminating one compound with only 0·75 per cent. of iron, the average content is seen to be 0·95 per cent.

$(C_{72}H_{112}N_{18}SO_{22})_4 + Fe - H_3 = 0{\cdot}86$ per cent. Fe.
$(C_{72}H_{112}N_{18}SO_{22})_3 + Fe - H_3 = 1{\cdot}14$ per cent. Fe.

As the iron was determined by simple ignition it would be expected that the amount found would exceed the theoretical amount somewhat; hence the first formula, assuming Lieberkühn's formula to be correct, would be more closely in accord with our results. The results obtained indicate further, that the iron albuminate is a much more stable compound than the copper albuminate, less liable to change and less readily affected by water.

(d) *Zinc Compounds.*

With zinc we made but a few experiments and those mainly to see whether the low percentage of iron found in the iron albuminate would be substantiated by a corresponding percentage of zinc in the zinc albuminate. Lieberkühn has prepared and analyzed a zinc albuminate, made by the action of zinc sulphate on a neutral solution of alkali albuminate, and he found the compound to contain 4·66 per cent. of zinc oxide.

Our preparations were made by the action of a similar zinc salt on a solution of purified and dialyzed albumin. Following are the results obtained with two preparations made from two distinct lots of albumin :

No.	Amt. sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·2424 gram.	0·0031 gram.	1·27	0·98
b	0·2833	0·0034	1·21	0·97
2*a*	0·2166	0·0023	1·06	0·83
b	0·2354	0·0025	1·08	0·86

The average of these two results shows a composition proportional to that found in the case of the iron albuminate and suggests plainly that if we have to deal in these cases with a single albuminate of constant composition, the percentage amount of metal is much smaller than formerly was supposed.

Further, the percentage of zinc found accords closely with the theoretical amount for a zinc albuminate formed on the type of the copper compound.

$$(C_{72}H_{112}N_{18}SO_{22})_4 + Zn - H_2 = 0{\cdot}99 \text{ per cent. Zn.}$$

(e) *Uranium Compounds.*

N. Kowalewsky* has recently called attention to the use of uranic or uranyl acetate as a reagent for albuminous matter, and has shown that it is not only a good precipitant of albumin at ordinary temperatures, but also that it is an extremely delicate one. Further, Kowalewsky states that the uranyl-albumin compound on ignition leaves a dark, olive green ash, composed of the green uranoso-uranic oxide, U_3O_8. A determination of the amount of this ash in several preparations showed 12·09 to 13·4 per cent., presumably of U_3O_8.

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* Essigsaures Uranoxyd, ein Reagens auf Albuminstoffe. Zeitschrift für Analytische Chemie, 1885, p. 551.

Our preparations were made by adding uranyl nitrate to the prepared albumin solution and washing the precipitated albuminate until all excess of uranium was removed. The uranium in the dried preparation was determined as uranoso-uranic oxide (U_3O_8) by simple ignition. The results show a fairly close agreement, but they are undoubtedly somewhat too high, owing to a small amount of adherent ash.

With $UO_2(NO_3)_2$.

No.	Amt. sub. taken.	Wt. U_3O_8.	Per cent. U_3O_8.	Per cent. U.
1*a*	0·5980 gram.	0·0324 gram.	5·41	4·59
b	0·5193	0·0281	5·41	4·59
2*a*	0·8219	0·0428	5·20	4·41
b	0·8081	0·0418	5·17	4·38
3*a*	0·4392	0·0251	5·71	4·84
b	0·5330	0·0303	5·68	4·81
4*a*	0·8183	0·0427	5·22	4·43
b	0·7576	0·0394	5·20	4·41
5	0·6985	0·0367	5·25	4·46
6*a*	0·4269	0·0247	5·78	4·90
b	0·5496	0·0313	5·70	4·83

These results plainly do not accord at all with Kowalewsky's. On the other hand they do agree fairly well with each other, and would seem to indicate a reasonably constant composition of the uranyl-albumin precipitate.

The average of the results obtained, accords most closely with the formula

$$(C_{72}H_{112}N_{18}SO_{22})_3 + U - H_6$$

which requires 4·73 per cent. U.

(f) *Mercury compounds.*

By the addition of an excess of mercuric chloride solution to an aqueous solution of egg-albumin, an albuminate of mercury is formed, insoluble in excess of the mercury salt. The compound can be easily filtered and admits of thorough washing with water. Rose first proved that the precipitate formed as above, is a compound of mercury with albumin, instead of a compound of the mercury salt with albumin as supposed by Bostock and Orfila.

We have made a few preparations of the albuminate by adding a moderately strong aqueous solution of mercuric chloride to portions of the dialyzed albumin solution and washing the precipitates thoroughly with water. The mercury in the albuminate was deter-

mined by ignition in a combustion tube with quick lime, with a posterior layer of calcium carbonate and sodium bicarbonate.*

The mercury distilled, was collected in water and after thorough washing with alcohol to remove hydrocarbons, etc., was dried and weighed.

Following are the results of our analyses of the several preparations made.

No.	Amt. Sub. taken.	Wt. Hg.	Per cent. Hg.
1*a*	0·8050 gram.	0·0226 gram.	2·80
b	0·8732	0·0274	3·13
2*a*	0·7637	0·0215	2·82
b	0·7357	0·0201	2·73
3*a*	0·5088	0·0150	2·96
b	0·6261	0·0167	2·66
4*a*	0·9152	0·0300	3·28
b	0·8503	0·0270	3·17
5*a*	0·8492	0·0218	2·56
b	0·8610	0·0237	2·75
6*a*	0·9674	0·0284	2·93

The average content of mercury is 2·89 per cent. The theoretical amount for $(C_{72}H_{112}N_{18}SO_{22})_4 + Hg - H_2$ is 3·00 per cent.

(g) *Silver compounds.*

Silver nitrate is a well known precipitant of albumin, and Lieberkühn,† many years ago, assigned to silver albuminate a definite formula, calling for 6·67 per cent. of silver oxide. The preparation made by him from egg-albumin was found to contain 6·55 per cent. of silver oxide = to 6·27 per cent. of Ag. Mulder,‡ likewise, working with alkali-albuminate, found that by neutralizing the solution as nearly as possible with acetic acid, and then precipitating with silver nitrate, the silver albuminate so prepared contained 6·14 per cent. of silver oxide.

Fuchs§, using ordinary egg-albumin instead of alkali-albuminate, found only half as much silver (3·28 per cent. Ag), while O. Loew,‖ working with purified egg-albumin, found still smaller percentages of silver in the albuminate made by him. Using an albumin

* See Fresenius, Quantitative Chemical Analysis.

† Poggendorff's Annalen, 1852, vol. clxii, p. 123.

‡ See Berzelius' Lehrbuch der Chemie, vol. ix, p. 49.

§ Annalen d. chem. u. Pharm., Band cli, p. 372.

‖ Pflüger's Archiv für Physiologie, Band xxxi, p. 393; Ueber Eiweiss und Pepton.

solution purified simply by three days' dialysis, Loew found that a 1 per cent. solution of silver nitrate gave no precipitate whatever in a 5 per cent. solution of albumin. On adding a little dilute sulphuric acid to the albumin solution, however, and then pouring the mixture into the silver solution a precipitate was obtained, which on thorough washing and drying was found to contain 2·17 per cent. of Ag; while a second preparation made by using a little less sulphuric acid contained 2·40 per cent. of Ag. By precipitating the albumin solution directly with a 5 per cent. solution of silver nitrate, without the addition of any acid, the albuminate was found to contain in one case 4·39 per cent. Ag, in a second case 3·91 per cent. Ag. Dissolving the freshly precipitated albuminate formed in this manner, in dilute ammonia and then reprecipitating it by the addition of dilute sulphuric acid to slight acid reaction, the albuminate was found to contain 4·64 per cent. of Ag.

Loew sees in these results a confirmation of Harnack's views as to the copper albuminates, and an assurance that the molecular weight of albumin corresponds to Lieberkühn's formula three times enlarged.

Using an albumin solution purified as in our previous experiments and adding to it a 10 per cent. solution of silver nitrate as long as a precipitate was formed, four distinct series of albuminates were made* representing four distinct preparations of egg-albumin. These were all washed free from silver and also from any adhering albumin, dried at 110° C. until of constant weight and the silver determined by simple ignition.

SERIES I.

No.	Amt. Sub. taken.	Wt. Ag.	Per cent. Ag.
1*a*	0·5900 gram.	0·0242 gram.	4·10
b	0·5625	0·0230	4·08
2*a*	0·5760	0·0230	4·11
b	0·7548	0·0305	4·04
3*a*	0·9005	0·0362	4·02
b	0·7973	0·0325	4·07

SERIES II.

1*a*	0·5859	0·0245	4·18
b	0·6967	0·0290	4·16
2*a*	0·9478	0·0385	4·06
b	0·6621	0·0270	4·07
3*a*	0·6455	0·0266	4·12
b	0·7000	0·0285	4·07

* The silver compounds were all made and analyzed by Mr. T. S. Bronson of this laboratory.

SERIES III.

1*a*	0·5860	0·0239	4·07
b	0·6949	0·0284	4·08
2*a*	0·7090	0·0290	4·09
b	0·9053	0·0374	4·13
3*a*	0·5980	0·0247	4·13
b	0·8000	0·0328	4·10

SERIES IV.

1*a*	0·6217	0·0300	4·88
b	0·6810	0·0331	4·86
2*a*	0·5509	0·0270	4·90
b	0·5626	0·0278	4·86
3*a*	0·6955	0·0396	5·69
b	0·7515	0·0430	5·72

The figures show a far smaller content of silver in all of the preparations than found by Lieberkühn or Mulder. In three of the series, there is seen a constancy of composition which is quite noticeable and, further, a close agreement with the second result obtained by Loew on adding a 5 per cent. solution of silver nitrate to the albumin. In the last series, however, the percentage of silver is somewhat higher, possibly owing to incomplete dialysis of the chlorides and phosphates from the albumin solution. These figures, however, are not much higher than the highest figures obtained by Loew.

A silver salt of albumin, of the composition $(C_{72}H_{112}N_{18}SO_{22})_2 + Ag_2 - H_2$ would contain 4·28 per cent. of Ag, and while our results certainly approximate to this figure, there is variation enough to indicate an equal possibility of a mixture of two or more compounds. With a molecule of the size of the albumin molecule, it is possible by doubling or otherwise, to obtain a formula corresponding to almost any percentage of metal found. And inasmuch as every variation* in the method of preparing the albuminate tends to alter its composition, it seems worse than useless at present, to lay much stress on the exact constitution of the silver albuminate. A large number of albuminates are of course possible, but until we know more definitely how to separate one from another, we have no guarantee of the simple nature of any one.

* Loew states that he has prepared a silver albuminate containing 10·7 per cent. Ag, corresponding nearly to 6 atoms of silver, and that it is possible to prepare albuminates still richer in silver.

Examining now, all of the results obtained, we find the following average composition of the albuminates studied :

Copper compound,	0·94	per cent.	Cu
Iron compound,	0·95	" "	Fe*
Zinc compound,	0·91	" "	Zn
Lead (neutral salt) compound,	2·56	" "	Pb
Uranyl compound,	4·60	" "	U
Silver compound,	4·09	" "	Ag†
Mercury compound,	2·89	" "	Hg

Accepting Lieberkühn's formula of albumin as correct, then the following formulæ accord most closely with the above percentages.

$(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2$	requires 0·96	per cent.	Cu
$(C_{72}H_{112}N_{18}SO_{22})_4 + Fe - H_2$	" 0·86	"	Fe
$(C_{72}H_{112}N_{18}SO_{22})_4 + Zn - H_2$	" 0·99	"	Zn
$(C_{72}H_{112}N_{18}SO_{22})_6 + Pb - H_2$	" 2·50	"	Pb
$(C_{72}H_{112}N_{18}SO_{22})_3 + U - H_6$	" 4·78	"	U
$(C_{72}H_{112}N_{18}SO_{22})_3 + Ag_4 - H_2$	" 4·28	"	Ag
$(C_{72}H_{112}N_{18}SO_{22})_4 + Hg - H_2$	" 3·00	"	Hg

We do not, however, lay much stress upon the accuracy of these formulæ. The results obtained in our study of these metallic compounds do by chance accord with them, and inasmuch as Loew and Harnack are disposed to treble Lieberkühn's formula for albumin, on the basis of the composition of the copper and silver albuminates, made by them respectively, we present our results as evidence that there are equally good grounds for quadrupling the above formula.

We believe, however, that with the majority of these albuminates it is possible to form a large variety of compounds with the same metal, by simply modifying the conditions of precipitation. This is evidenced by Loew's results with silver albuminate and our own with lead and copper, and since a great variety of compounds are possible, it is equally possible that in many cases we may have to do with mixtures of such compounds, which would account for the great variability in composition noticed in some of the albuminates and for the lack of agreement in the results obtained by different workers. Coupled with this, in some cases, is the undoubted tendency of the compounds to dissociation.

II. *Myosin.*

The myosin employed was prepared from ox flesh, by extraction with a 15 per cent. solution of ammonium chloride, after the tissue

* Excepting one very low result. † Excepting the last series of compounds.

had been thoroughly freed from salts and soluble albumin by long continued extraction with water. The myosin was separated from the ammonium chloride solution by dialysis, being obtained in this manner as a semi-gelatinous mass, readily soluble in salt solutions. In order to form compounds with the various metals, it was found best to use a solution of myosin in 5 per cent. ammonium chloride, the metallic compound when formed being washed with water until the washings gave no reaction for chlorine with silver nitrate. The compounds were then dried, first at 100° C., then at 110° C., until of constant weight. Control experiments with the metallic salt and ammonium chloride alone, invariably failed to give any precipitate whatever.

No systematic attempt has apparently been made to study any of the metallic compounds of myosin; in fact, few statements are to be found regarding the existence of such compounds. Danilewsky* some time ago, showed that myosin would combine with free mineral acids, uniting with them so that with tropæolin 00 no reaction for free acid could be obtained. With strong bases, however, according to Danilewsky, myosin does not probably combine, and the statement is further made that a small amount of calcium oxide ordinarily exists loosely combined with myosin, which calcium by coagulation of the myosin is liberated. Further, Danilewsky found that on adding platinum chloride in excess, to a dilute hydrochloric acid solution of myosin, a myosin-platinum chloride compound was precipitated, which after washing with water and alcohol and then drying at 100–105° C., contained 9·46 per cent. of platinum and 7·26 per cent. of chlorine. With copper, iron and similar salts we have not been able to obtain any precipitate in a hydrochloric acid solution of myosin. By adding, however, a solution of a metallic salt of such a nature that it does not react with ammonium chloride, to an ammonium chloride solution of myosin, a precipitate is produced, which as our experiments show, is ordinarily a compound of myosin with the metal or metallic oxide. This is readily seen by adding either zinc sulphate or ferric chloride to such a solution of myosin and then washing the precipitates with water, until the washings give no reaction for chlorine or for sulphuric acid. On now warming the iron precipitate with dilute nitric acid, a solution will be obtained, giving a distinct iron reaction but no reaction with silver nitrate for chlorine. Similarly on warming the zinc pre-

* Zeitschrift für physiologische Chemie, v, p. 160.

cipitate with hydrochloric acid, the solution gives no reaction for sulphuric acid with barium chloride.

With cupric sulphate and cupric acetate the same is ordinarily true. It is possible, however, to prepare a myosin-copper compound in which cupric sulphate appears to unite directly with the myosin.

(*a.*) *Copper compounds.*

By adding either cupric sulphate or cupric acetate to a neutral ammonium chloride solution of myosin, a heavy greenish colored precipitate is obtained, which when freshly formed and after thorough washing with water, so that the washings are entirely free from chlorine and from copper, shows the following reactions. It is insoluble in moderately strong nitric, hydrochloric or sulphuric acid. The compound, however, is immediately broken up by the action of acids, the copper being completely removed, leaving the myosin as an insoluble residue having in the case of nitric acid a yellow color, and in the case of hydrochloric and sulphuric acids a white color. In acetic acid, the compound is more soluble, first, however, becoming semi-gelatinous. In ammonium hydroxide, the compound dissolves slowly or partially, taking on a blue color. In dilute sodium hydroxide, the compound swells up, takes on a purple color, but does not dissolve. In dilute sodium carbonate, the substance is likewise insoluble, but swells up and turns of a bluish color.

Following are the results of the analyses of the various preparations made. The compounds were in every case composed simply of the metallic oxide and myosin. Copper was determined, as in the case of the albumin compounds, by simple ignition and weighing as oxide. In order to ascertain how much ash was retained by the myosin compound, a few duplicate determinations of copper were made by dissolving the oxide after ignition, and precipitating the copper as sulphide and weighing as subsulphide, after ignition with a little pure sulphur in a current of hydrogen gas.

SERIES I.

With $CuSO_4$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·5426 gram.	0·0074 gram.	1·08	0·0056 gram.	0·81
b	0·7176	0·0097	1·07	0·0073	0·80
2*a*	0·6359	0·0087	1·08	0·0071	0·88
b	0·5738	0·0078	1·08	0·0061	0·83

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
3*a*	0·5428 gram.	0·0072 gram.	1·05	0·0051 gram.	0·73
b	0·6241	0·0082	1·05	0·0063	0·80
4*a*	0·5771	0·0109	1·50		
b	0·4455	0·0081	1·43		
5	0·3527	0·0046	1·02		
6*a*	0·4401	0·0062	1·11		
b	0·5708	0·0082	1·18		

Series II.

With $CuSO_4$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu.
1*a*	0·7425 gram.	0·0059 gram.	0·79	0·63
b	0·7463	0·0060	0·81	0·64
2*a*	0·7785	0·0057	0·73	0·57
b	0·7767	0·0056	0·72	0·58
3*a*	0·5488	0·0052	0·94	0·74
b	0·4336	0·0041	0·94	0·73
4*a*	0·9477	0·0085	0·89	0·74
b	0·8150	0·0074	0·91	0·73

With $Cu(C_2H_3O_2)_2$.

5*a*	0·8522	0·0133	1·56	1·24
b	0·8466	0·0138	1·62	1·29
6*a*	0·6589	0·0124	1·88	1·50
b	0·6572	0·0123	1·87	1·50
7*a*	0·8673	0·0156	1·79	1·42
b	0·7120	0·0129	1·81	1·44

Series III.

With $CuSO_4$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu.
1*a*	0·7218 gram.	0·0171 gram.	2·37	1·88
b	0·6584	0·0161	2·44	1·94
2*a*	0·7564	0·0168	2·22	1·77
b	0·6817	0·0156	2·29	1·83
3*a*	0·7820	0·0189	2·41	1·91
b	0·6213	0·0154	2·48	1·97
4*a*	0·7137	0·0144	2·01	1·61
b	0·5863	0·0124	2·11	1·68
5*a*	0·7732	0·0164	2·12	1·68
b	0·7407	0·0161	2·18	1·74
6*a*	0·7447	0·0183	2·45	1·96
b	0·7429	0·0188	2·53	2·01

With $Cu(C_2H_3O_2)_2$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu.
7*a*	0·7360 gram.	0·0161 gram.	2·18	1·78
b	0·7088	0·0154	2·17	1·78
8*a*	0·7789	0·0174	2·24	1·78
b	0·8111	0·0181	2·23	1·77
9*a*	0·7376	0·0169	2·29	1·81
b	0·6155	0·0138	2·24	1·78
10*a*	0·7815	0·0191	2·44	1·94
b	0·8774	0·0213	2·43	1·93
11*a*	0·8048	0·0170	2·11	1·67
b	0·8308	0·0174	2·10	1·67
12*a*	0·6780	0·0150	2·21	1·75
b	0·7695	0·0168	2·18	1·74

Comparing these results with one another, there is to be seen a very noticeable lack of agreement in composition, and further it is to be seen that the myosin-copper compound has on an average a somewhat higher content of copper than the albumin-copper precipitate. The average composition of all the copper myosins shows about 1·42 per cent. of Cu, and deducting 0·25 per cent. of ash, the average content of Cu would be 1·17 per cent. Examining the individual results, it is apparent that the compounds made from the same myosin solution are approximately, at least, the same in composition and without doubt the difference in the composition of compounds made from different myosin solutions is due to variation in the concentration of, and possibly also in the reaction of, the myosin-containing fluid. It would appear as if variations in the conditions of precipitation made a greater difference in the case of the myosin-copper compounds than in the compounds of copper with albumin. Several times, also, we have found that our myosin-copper precipitate contained sulphuric acid, even after thorough washing and when the wash-water was proved to be entirely free from any reaction with barium chloride.

One such compound, after drying at 110° C., was analyzed with the following results:

0·7240 gram substance* gave 0·0848 gram $BaSO_4$ = 1·63 per cent. SO_3.
0·8420 gram substance gave 0·0881 gram $BaSO_4$ = 1·73 per cent. SO_3.
0·4705 gram substance† gave 0·0084 gram CuO = 1·78 per cent. CuO.
0·6011 gram substance gave 0·0107 gram CuO = 1·78 per cent. CuO.

* Roasted and then ignited with pure sodium carbonate, the residue dissolved in hot water acidified with hydrochloric acid and precipitated with barium chloride.

† Ignited, the residue dissolved in nitric acid, precipitated with hydrogen sulphide, etc.

The molecular weight of CuO and SO_3 being the same, it is evident that the two are present in just the proportion to form cupric sulphate.

b. Iron compounds.

By adding a solution of ferric chloride to an ammonium chloride solution of myosin, a semi-gelatinous precipitate is formed of a reddish yellow color, and consisting of a combination of myosin and oxide of iron. The compound when thoroughly washed contains no chlorine. When freshly precipitated, it is partially soluble in dilute ammonium hydroxide, as also in sodium hydroxide, the residue becoming gummy or gelatinous and brownish yellow in color. It swells up in sodium carbonate, but is insoluble. In nitric acid the compound turns yellow, but is wholly insoluble and does not swell up. In hydrochloric and also in sulphuric acid the compound is likewise insoluble. In acetic acid, however, it is soluble completely, forming a semi-gelatinous fluid. In this, as in other metallic compounds of myosin, acids simply dissolve out the metal and then exert their usual action on the myosin. The various preparations, washed free from iron and chlorine, and dried at 110° C. were analyzed with the following results:

SERIES I.

No.	Amt. Sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·7046 gram.	0.0194 gram.	2·76	1·93
a	0·7021	0·0193	2·74	1·92
2*a*	0·3762	0·0128	3·40	2·38
b	0·3623	0·0121	3·33	2·33
3*a*	0·5837	0·0141	2·42	1·69
b	0·5618	0·0137	2·43	1·70
4*a*	0·5488	0·0177	3·25	2·26
b	0·6088	0·0197	3·24	2·26
5*a*	0·4761	0·0140	2·95	2·06
b	0·3381	0·0100	2·95	2·07
6*a*	0·5101	0·0173	3·40	2·37
b	0·5927	0·0194	3·28	2·29

SERIES II.

1	0·4847	0·0188	3·87	2·70
2	0·3200	0·0115	3·59	2·51
3	0·4118	0·0153	3·72	2·59
4	0.5205	0·0184	3·53	2·45

No.	Amt. Sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
5*a*	0·4551 gram.	0·0167 gram.	3·68	2·57
b	0·3668	0·0137	3·73	2·61
6*a*	0·3783	0·0138	3·64	2·53
b	0·3675	0·0131	3·57	2·50
7*a*	0·4284	0·0143	3·34	2·33
b	0·4041	0·0136	3·37	2·36

SERIES III.

With a large excess of ferric chloride.

1*a*	0·4385	0·0269	6·13	4·29
b	0·6939	0·0436	6·27	4·38

In none of these preparations was there any attempt made to add a definite amount of ferric chloride, but the iron salt was added until a good precipitate was obtained. Undoubtedly, the amount of iron salt added, modifies materially the composition of the compound. In series III it is seen that the content of iron is about double the average amount contained in the other preparations. The average amount of iron (Fe) in the first two series of compounds is 2·29 per cent.

c. Zinc compounds.

With zinc sulphate, myosin is thrown down from its ammonium chloride solution as a heavy gelatinous precipitate. Like the iron compound it is partially soluble in sodium and ammonium hydroxides, swelling up to a gelatinous mass. It is insoluble in nitric, hydrochloric and sulphuric acids, but is partially soluble in acetic acid. In composition, it is seen to be very closely allied to the zinc albuminate. Following are the results obtained by analysis of the dried compounds:

SERIES I.

No.	Amt. Sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·6108 gram.	0·0049 gram.	0·81	0·64
b	0·6619	0·0055	0·83	0·66
2*a*	0·6611	0·0047	0·71	0·57
b	0·8006	0·0059	0·73	0·59
3*a*	0·4666	0·0046	0·99	0·79
b	0·4494	0·0044	0·99	0·79
4*a*	0·5936	0·0048	0·80	0·64
b	0·6926	0·0055	0·79	0·63

SERIES II.

No.	Amt. Sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·6258 gram.	0·0051 gram.	0·82	0·65
b	0·6618	0·0054	0·81	0·65
2*a*	0·5315	0·0064	1·21	0·97
b	0·5485	0·0065	1·18	0·94
3*a*	0·7925	0·0064	0·81	0·65
b	0·6913	0·0057	0·83	0·66
4*a*	0·4858	0·0062	1·27	1·02
b	0·5318	0·0066	1·24	0·99
5*a*	0·6635	0·0056	0·85	0·68
b	0·6504	0·0050	0·76	0·61
6*a*	0·6263	0·0055	0·87	0·70
b	0·6639	0·0059	0·88	0·71

The average content of zinc (Zn) is 0·72 per cent. Unlike the iron and copper compounds, there is here less variation in the composition of the various preparations.

d. Nickel and cobalt compounds.

The extremely low percentage of zinc in the zinc-myosin compounds, as contrasted with the iron in the iron compounds, led us to make a nickel and cobalt preparation for the sake of comparison. The results, in both cases, accord more nearly with those of the iron compound, for although containing a higher percentage of metal than the latter, it was necessary to prepare them both under just such conditions as in the iron compound led to the highest percentage of iron, viz: a large excess of the precipitant. Following are the analytical results obtained with both substances:

With $Ni(NO_3)_2$.

No.	Amt. Sub. taken.	Wt. NiO_2.	Per cent. NiO_2.	Per cent. Ni.
1*a*	0·6165 gram.	0·0045 gram.	7·29	4·71
b	0·7016	0·0051	7·26	4·70

With $CO(NO_3)_2$.

1*a*	0·6479	0·0064	9·95	6·45
b	0·6696	0·0065	9·70	6·28
2*a*	0·6451	0·0056	8·75	5·67
b	0·6842	0·0060	8·84	5·72

e. Uranium compounds.

The addition of uranyl nitrate to an ammonium chloride solution of myosin produces a heavy gelatinous precipitate of a uranyl-myosin compound, which in solubility resembles the other myosin preparations. Washed free from excess of uranyl nitrate and from ammonium chloride and then dried at 110° C., the various preparations yielded on analysis the following results, the uranium being determined by simple ignition and weighing as uranoso-uranic oxide:

SERIES I.

No.	Amt. Sub taken.	Wt. U_3O_8.	Per cent. U_3O_8.	Per cent. U.
1*a*	0·6873 gram.	0·0489 gram.	7·67	6·51
b	0·6886	0·0526	7·69	6·53
2*a*	0·7081	0·0592	8·36	7·09
b	0·7689	0·0655	8·51	7·22
3*a*	0·6418	0·0586	9·13	7·75
b	0·7809	0·0715	9·16	7·78
4*a*	0·6621	0·0541	8·17	6·93
b	0·7455	0·0608	8·15	6·91
5*a*	0·7525	0·0663	8·81	7·48
b	0·6964	0·0615	8·83	7·50
6*a*	0·7208	0·0590	8·18	6·94
b	0·7439	0·0607	8·15	6·91
7*a*	0·6648	0·0527	7·92	6·72
b	0·7101	0·0565	7·96	6·76

SERIES II.

1*a*	0·8736	0·0837	9·58	8·13
b	0·8570	0·0820	9·57	8·12
2*a*	0·5857	0·0593	10·12	8·59
b	0·7088	0·0715	10·09	8·56
3*a*	0·5715	0·0568	9·94	8·44
b	0·6958	0·0688	9·89	8·40
4*a*	0·6929	0.0556	8·02	6·82
b	0·7667	0·0615	8·02	6·82
5*a*	0·8081	0·0874	10·88	9·23
b	0·8272	0·0901	10·89	9·24
6*a*	0·7034	0·0553	7·86	6·67
b	0·6810	0·0540	7·92	6·72

These results show a variation in the content of uranium, amounting to nearly 3 per cent. (6·51–9·24 per cent.) Further, a compari-

son of the two series shows plainly that there is something in the nature of the second myosin solution, which tends to raise the content of uranium in the uranyl compounds; probably, the greater concentration or dilution of the solution. Evidently, then, the composition of the compound is, in part at least, determined by the conditions under which the uranyl salt and the myosin solution are brought together. The average amount of uranium contained in the preparations is 7·49 per cent.

f. Mercury compounds.

By adding a solution of mercuric chloride to an ammonium chloride solution of myosin, a heavy gelatinous precipitate is formed which soon changes to a flocculent one. Freed from the excess of mercury salt and ammonium chloride, the compound is found to be entirely free from chlorine. The substance is somewhat soluble in sodium hydroxide, swelling up first and then gradually dissolving. Dried at 110° C. and then analyzed, the following results were obtained. The mercury was determined as already described under mercury albuminate.

SERIES I.

No.	Amt. Sub. taken.	Wt. of Hg.	Per cent. Hg.
1*a*	0·7609 gram.	0·0166 gram.	2·18
b	1·2911	0·0268	2·07
2*a*	1·2386	0·0270	2·17
b	0·9525	0·0198	2·07
3*a*	1·1856	0·0218	1·84
b	0·9261	0·0173	1·86
4*a*	1·3504	0·0257	1·90
b	0·9332	0·0178	1·90

SERIES II.

1	0·8917	0·0241	2·70
2	1·1196	0·0310	2·77
3*a*	0·9537	0·0271	2·84
b	0·8813	0·0259	2·93
4	0·9209	0·0270	2·93
5	0·8352	0·0241	2·89
6	0·8564	0·0217	2·53
7*a*	1·0696	0·0344	3·22
b	0·8550	0·0264	3·09

The average content of mercury (Hg) is 2·43 per cent. The results of each series show a fairly close agreement, but the two series do not compare with each other at all. Thus, the average amount of mercury in the compounds of the first series is 1·99 per cent., while in the second series the average amount rises to 2·87 per cent. This is another good illustration of the influence of the strength of the solution on the composition of the precipitate, and as in this case the presence of any ash could not interfere with the ultimate result, since the mercury was separated by distillation, it follows that the apparently higher content of mercury in the second series must be due to combination of the myosin with a larger amount of the metal. Further, it has been claimed* that in the case of the silver albuminate, it is possible under certain circumstances for the albuminate, when formed in a concentrated solution, to inclose a variable amount of albumin mechanically, and thus the apparent percentage of silver in the albuminate be reduced. If such was true of the myosin-mercury compounds, a far greater variation would be expected in the percentage of mercury in the different preparations of the same series.

The following table of comparisons shows the average content of metal in the albuminates formed from the two kinds ot proteid matter.

	Egg-albumin.		Myosin.	
Copper compound,	0·94 per cent.	Cu	1·17 per cent.	Cu
Iron "	0·95	Fe	2·29	Fe
Zinc "	0·91	Zn	0·72	Zn
Uranyl "	4·60	U	7·49	U
Mercury "	2·89	Hg	2·43	Hg
Lead "	2·56	Pb	----	----
Silver "	4·09	Ag	----	----
Nickel "			4·70	Ni
Cobalt "			6·03	Co

Apparently, the two forms of albuminous matter, the albumin and globulin, do not form corresponding compounds with the metallic salts experimented with.

* See Loew, Pflüger's Archiv für Physiologie, Band xxxi, p. 393.

Egg-Albumin and Albumoses. By R. H. Chittenden and Percy R. Bolton, Ph.B.

Ever since the albumose bodies were first separated from the products of fibrin digestion* with pepsin-hydrochloric acid, it has been our intention, already expressed, to subject the various individual albumins† to the action of purified pepsin under like conditions, and thus ultimately to acquire a comparative knowledge of the albumose bodies obtainable from these different sources. Already the albumose bodies from fibrin and the globuloses‡ have been subjected to a careful study and we present here the result of a study of the albumoses from egg-albumin.

In doing this, we have to report at the same time, the results of a study of the composition of egg-albumin itself. For we have made it a rule, in the series of experiments shortly to be described, to analyze a sample of each lot of albumin prepared for digestion. In this manner we have obtained data for a direct comparison of composition between the original sample of albumin and the products formed by its digestion. This we have deemed of considerable importance, for the data so obtained may throw considerable light on the nature of the changes involved in the formation of the albumoses; particularly, as to whether they are hydrolytic in their nature.

Four distinct samples of albumin were prepared, three of which were prepared in large quantities and served as material for the subsequent digestions.

Albumin A.

This was a preparation of coagulated egg-albumin, prepared especially with the view of obtaining a product wholly free from globulin. The method employed was essentially that recommended by Hammarsten.§ The whites of 120 eggs were freed from the yolks, then

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* W. Kühne and R. H. Chittenden, Ueber Albumosen, Zeitschrift für Biologie, Band xx, p. 11.

† W. Kühne and R. H. Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskörper, Zeitschrift für Biologie, Band xix, p. 159.

‡ W. Kühne and R. H. Chittenden, Globulin und Globulosen, Zeitschrift für Biologie, Band xxii, p. 409.

§ See K. V. Starke, Beiträge zur Kenntniss des Serum- und Eialbumins, Jahresbericht für Thierchemie, 1881, p. 18.

finely divided by shaking with glass, the fluid mixed with an equal volume of water or more, then shaken vigorously with air and finally filtered through cloth. The solution so obtained, was then saturated with crystals of magnesium sulphate at 20° C., for the complete removal of the globulin. The mixture was filtered through paper and the clear filtrate saturated with sodium sulphate. The precipitated albumin was then filtered and washed with a saturated solution of sodium sulphate, after which it was dissolved in water and dialyzed in running water until the magnesium and sodium sulphates were entirely removed. The fluid was then again filtered and the albumin finally coagulated by being poured into eight litres of boiling water, slightly acidified with acetic acid. The great bulk of the coagulum so obtained was at once placed in four litres of 0·4 per cent. hydrochloric acid, while a small sample for analysis was washed with 95 per cent. alcohol, finally with absolute alcohol and then dried, first at 100° C., and finally at 106° C., *in vacuo*, until of constant weight. The following table shows the results of the analysis of the product. The various determinations were made as described in the previous articles on these subjects, the sulphur being determined by fusion with potassium hydroxide and potassium nitrate in a silver crucible, according to the method designated by Hammarsten* as 1*a*.

Albumin B.

This albumin was prepared from the whites of 120 eggs by a somewhat different method. The albumin solution, after dilution with water, was made very distinctly acid with acetic acid, and the heavy precipitate of globulin, after it had well settled, removed by filtration. The acid fluid was then made exactly neutral with sodium carbonate and again filtered; it was then thymolized and dialyzed in running water for eight days. A little globulin, not precipitated by the acetic acid, was found in the bottom of the dialyzers when the salts had diffused out. This was filtered off and the perfectly clear fluid evaporated at 35–45° C., to perfect dryness. A sample of this preparation was ground fine, dried at 106° C. *in vacuo* and analyzed. It is perhaps questionable, whether all of the globulin is removed by this method. The precipitate with acetic acid was quite heavy, and as H. Dillner† has recently shown that the amount of globulin in egg-albumin, as

* See Zeitschrift für physiolog. Chemie, Band ix, p. 289.

† Ueber die Globuline im Hühnereiweiss, Jahresbericht für Thierchemie, 1885, p. 31.

ANALYSIS OF ALBUMIN A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
I	0·3938	0·2455	6·92	0·7517	52.05	....	....	.....	.....	.....	....	.....	....
II	0·6406	0·4007	6·95	1·2247	51.98	....	....	.....	.....	.....	....	.....	....
III	0·6133	.....	....	.....	....	80·5	8·2	749·1	15·78	.....	....	.....	....
IV	0·6189	.....	....	.....	....	79·8	6·8	754·5	15·68	.....	....	.....	....
V	0·5209	.....	....	.....	....	66·6	6·8	766·9	15·81	.....	....	.....	....
VI	0·5751	.....	....	.....	....	....	....	.....	.....	0·0798	1·90	.....	....
VII	0·5926	.....	....	.....	....	....	....	.....	.....	0·0833	1·93	.....	....
VIII	0·6417	.....	....	.....	....	....	....	.....	.....	0·0916	1·96	.....	....
IX	0·6366	.....	....	.....	....	....	....	.....	.....	.....	....	0·0023	0·37

Percentage composition of the ash-free substance.

									Average.
C	52·24	52·18	....	....	....	....	....	....	*52·21*
H	6·95	6·98	....	....	....	....	....	....	*6·96*
N	....	....	15·88	15·74	15·85	....	....	....	*15·80*
S	....	....	....	....	....	1·91	1·94	1·97	*1·94*
O	....	....	....	....	....	....	....	....	*23·09*
									100·00

X	0·4430	-----	----	-----	----	---	----	- -	----	------	----	0·0049	1·10
XI	0·4901	-- --	----	-----	----	----	----	-----	----	------	----	0·0055	1·12

Percentage composition of the ash-free substance.

										Average.
C	52·28	52·39	----	----	----	---	----	----	----	*52·33*
H	7·00	6·97	----	----	----	----	----	----	----	*6·98*
N	----	----	15·87	15·91	----	----	----	----	----	*15·89*
S	----	----	----	----	1·78	1·93	1·74	1·85	1·84	*1·83*
O	----	----	----	----	----	----	----	----	----	*22·97*
										100·00

determined by the magnesium sulphate method, never reaches 1 per cent., but averages only 0·667 per cent., it seems probable that the greater portion is separated by the acetic acid. Further, Dillner has found that on the dialysis of a neutralized egg-albumin solution, the matter which separates out after a few days dialysis, is only in part globulin, but consists, in addition, of a somewhat insoluble body rich in sulphur. Hence, the substance which separated in our dialyzers, after precipitation with acetic acid and neutralization, may not have been composed wholly of globulin. The following table shows the composition of the uncoagulated albumin B.

Albumin C.

This preparation was much the same as albumin B, except that it was finally coagulated. Globulin was separated by acetic acid, the filtrate neutralized, again filtered and the fluid dialyzed in running water until all soluble salts were removed. The albumin was then coagulated by being poured into a large volume of boiling water acidified with acetic acid. A sample, after drying at 106° C. *in vacuo*, was found to have the composition shown in the accompanying table.

Albumin D.

This sample of albumin was prepared in exactly the same manner as albumin A; the globulin removed by magnesium sulphate, the albumin precipitated by sodium sulphate and after dialysis, coagulated as already described. Its composition is shown in the following table.

Comparing now, the results of the analysis of these four samples of albumin, it is seen that the first three agree almost exactly in composition, while the fourth shows a somewhat lower content of carbon.

	A	*B*	*C*	*D*	*Average.*
C	52·21	52·33	52·46	51·74	52·18
H	6·96	6·98	7·00	6·81	6·93
N	15·80	15·89	15·88	15·68	15·81
S	1·94	1·83	1·69	2·02	1·87
O	23·09	22·97	22·97	23·75	23·21
Ash	0·37	1·11	0·17	0·45	

Further, the coagulated products (A and C) do not differ at all in composition from the non-coagulated albumin B.

Schützenberger,* as a result of his work on proteid matter, ascribed

* Bulletin de la Société Chimique de Paris, T. 23 et 24.

ANALYSIS OF ALBUMIN C.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
I	0·2930	0·1850	7·01	0·5633	52·42	....	....	....	....	.....	....	....	....
II	0·6268	0·3933	6·97	1·2033	52·35	....	....	....	....	.....	....	....	....
III	0·5936	.....	....	.....	....	79·5	15·7	752·4	15·74	.....	....	....	....
IV	0·3742	.....	....	.....	....	50·8	16·3	754·8	15·98	.....	....	....	....
V	0·5979	.....	....	.....	....	....	....	....	....	0·0786	1·69	....	....
VI	0·7189	.....	....	.....	....	....	....	....	....	0·0872	1·68	....	....
VII	0·7333	.....	....	.....	....	....	....	....	....	....	....	0·0013	0·17

Percentage composition of the ash-free substance.

							Average.
C	52·49	52·43	....	....	....	....	*52·46*
H	7·02	6·98	....	....	....	....	*7·00*
N	....	....	15·77	16·00	....	....	*15·88*
S	....	....	....	....	1·69	1·68	*1·69*
O	....	....	....	....	....	....	*22·97*
							100·00

ANALYSIS OF ALBUMIN D.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·6009	0·3695	6·83	1·1340	51·46	----	----	-----	----	-----	----	-----	-----
II	0·4564	0·2765	6·73	0·8630	51·56	----	----	-----	----	-----	----	-----	-----
III	0·5094	-----	----	-----	----	65·1	6·0	756·5	15·63	-----	----	-----	-----
IV	0·7652	-----	----	-----	----	96·0	4·7	765·5	15·60	-----	----	-----	-----
V	0·9402	-----	----	-----	----	----	----	-----	----	0·1379	2·01	-----	-----
VI	0·6664	-----	----	-----	----	----	----	-----	----	0·0982	2·02	-----	-----
VII	1·2907	-----	----	-----	----	----	----	-----	----	-----	----	0·0058	0·45
VIII	1·0975	-----	----	-----	----	----	----	-----	----	-----	----	0·0051	0·46

Percentage composition of the ash-free substance.

							Average.
C	51·69	51·79	----	----	----	----	*51·74*
H	6·86	6·76	----	----	----	----	*6·81*
N	----	----	15·69	15·67	----	----	*15·68*
S	----	----	----	----	2·02	2·03	*2·02*
O	----	----	----	----	----	----	*23·75*
							100·00

to albumin the formula $C_{240}H_{392}N_{65}O_{75}S_3$, which requires a content of carbon not far different from the average of our results, but which on the other hand demands a content of nitrogen nearly 1 per cent. higher than we found. The well-known Lieberkühn's formula requires 53·59 per cent. of carbon, or 1 per cent. more than was found in our highest result. Harnack's formula for albumin,* $C_{204}H_{322}N_{52}O_{66}S_2$, with a molecular weight of 4618, based on a study of the copper compounds of albumin, requires too high a content of carbon and altogether too low a percentage of sulphur. Lieberkühn's formula requires 1·98 per cent. of sulphur, while Harnack's formula requires only 1·39 per cent.; and as this was one of the main points on which Harnack based his formula, it is well to consider it. Our lowest result on sulphur is 1·69 per cent., and as the other three show a close agreement, it is probable that the former is somewhat too low. The average of our results, however, is but 0·04 per cent. higher than found by Lieberkühn. O. Loew† has recently considered this question, and he found on determining the sulphur in coagulated egg-albumin by a modification of Piria and Schiff's method, 1·70 and 1·87 per cent. of sulphur respectively. O. Nasse,‡ likewise, found in coagulated albumin a content of 1·72 per cent. of sulphur, and lastly, Hammarsten§ found in non-coagulated albumin 1·93 per cent. of sulphur. There would seem to be plenty of confirmatory evidence, therefore, that the content of sulphur in egg-albumin is much larger than indicated by Harnack's formula.

The nitrogen, as determined in our preparations, is seen to be somewhat higher than found by Hammarsten, with whose results in other respects ours most closely correspond. Dumas, however, found nearly the same percentage of nitrogen as contained in our preparations. The accompanying table of analyses shows the average of our results, compared with those of others.

Albumoses.

Three distinct digestions of albumin (preparations A, B and C) were made with pepsin-hydrochloric acid, and the albumose bodies isolated. In this way it was possible to prepare the bodies under somewhat different conditions, and to notice the influence, if any, on the nature and composition of the products. The pepsin-hydrochloric acid used in two of the digestions was prepared with a special view

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* Zeitschrift für Physiolog. Chemie, Band v, p. 207.

† Pflüger's Archiv für Physiologie, Band xxxi, p. 395.

‡ Jahresbericht für Thierchemie, 1873, p. 13. § Ibid, 1881, p. 19.

ANALYSES OF EGG-ALBUMIN.

	1	2	3	4	5	6	7	8
C	53·5	53·51	53·87	52·25	52·18	53·59	52·58	53·01
H	7·0	7·03	7·10	6·90	6·93	6·95	7·15	6·98
N	15·5	15·61	15·77	15·25	15·81	15·65	16·61	15·76
S	1·6	1·83	-----	1·93	1·87	1 98	1·75	1·89
O	----	-----	-----	-----	--- -	21·83	21·91	22·86

1. As analyzed by Mulder, Journal für prakt. Chemie, Band xliv, p. 509.
2. As analyzed by Lieberkühn. Poggendorff's Annalen, 1852, p. 117.
3. As analyzed by Dumas and Cahous. Ann. chem. Phys. (3) vi, p. 408.
4. As analyzed by Hammarsten. Jahresbericht für Thierchemie, 1881, p. 19.
5. Average of the results by Chittenden and Bolton.
6. Theoretical composition according to Lieberkühn's formula ($C_{72}H_{112}N_{18}SO_{22}$).
7. Theoretical composition according to Schützenberger's formula ($C_{240}H_{392}N_{66}O_{78}S_2$)
8. Theoretical composition according to Harnack's formula ($C_{204}H_{322}N_{52}O_{66}S_2$).

to removing all traces of albumose bodies, formed by the self-digestion of the mucous membrane, and was prepared as follows: 700 grams of mucous membrane from the cardiac portion of six pigs' stomachs, freed from the muscularis, were finely divided and warmed at 40° C. for fourteen days, in two and a half litres of 0·5 per cent. hydrochloric acid. At the end of this time, all albumose bodies presumably having been converted into peptone, the solution was filtered from the residue of nuclein, antialbumid, etc., and the filtrate saturated with ammonium sulphate. The precipitate, consisting mainly of pepsin, with perhaps some albumose, was filtered off, washed with a saturated solution of ammonium sulphate, and then dissolved in two litres of 0·2 per cent. hydrochloric acid. The acid solution was then thymolized and dialyzed in running water, until the ammonium sulphate was entirely removed. On opening the dialyzing tubes, quite a precipitate was found, which on being dissolved in 0·2 per cent. hydrochloric acid showed marked proteolytic action. The filtrate also, on being acidified, showed vigorous digestive power. These two solutions of purified pepsin were used in the digestion of two of the albumins, while with the third a pure glycerin extract of pepsin was employed.

The general method of procedure, both in the digestions themselves and in the separation of the various albumoses, was much the same as that previously employed by Kühne and Chittenden.

Digestion of Albumin A.

The albumin, as previously described, was placed in four litres of 0·4 per cent. hydrochloric acid and the mixture raised to a temperature of 45° C. Then 600 c. c. of the purified pepsin-hydrochloric acid solution were added and the mixture kept at a temperature of 45° C. for three hours, after which it was neutralized with sodium hydroxide and filtered. The pepsin solution, although quite active, did not act very vigorously on the coagulated albumin. The neutralization precipitate, therefore, together with the unaltered albumin, was again treated with a fresh quantity of the pepsin-hydrochloric acid, under like conditions as the preceding, for four hours. The two neutralized fluids were then united and treated together. The total volume was about six litres. The clear fluid was saturated in the cold with crystals of sodium chloride, by which a precipitate was obtained, which from analogy should consist of proto-, dys- and heteroalbumose.

In making these separations of the albumose bodies, we intentionally avoided raising the temperature of the fluid above 45° C., for fear that heat might induce some change in the character of the bodies; hence the first neutralized fluid was saturated directly with salt, in spite of its large volume, and the bodies were ultimately all separated without having been exposed to a temperature higher than that above-mentioned. The use, however, of such a large quantity of rock salt introduced into the solutions some calcium sulphate, which adhered very tenaciously to the albumose bodies and thus unavoidably raised the content of ash in the preparations.

The precipitate produced by the addition of sodium chloride in substance was filtered, washed with a saturated solution of sodium chloride, then extracted successively with a ten per cent. solution of sodium chloride, a five per cent. solution of the same salt, and lastly with water. The residue remaining undissolved after these successive treatments with dilute salt solutions and water, presumably consisted of dysalbumose, while the solutions contained a body precipitable by acetic acid and soluble in excess, and also precipitable by potassium ferrocyanide; presumably protoalbumose together with heteroalbumose. The original salt-saturated filtrate contained all of the deuteroalbumose, together with considerable protoalbumose and some heteroalbumose.

A. Protoalbumose.

The five and ten per cent. sodium chloride solutions of the first salt precipitate, together with the aqueous solution of the same, were united and then dialyzed in running water for removal of the heteroalbumose. The solution, partially freed from the latter, was concentrated somewhat and the protoalbumose again precipitated by saturating the solution with sodium chloride. This precipitate was again dissolved in water, dialyzed until the greater portion of the salt was removed, the solution then concentrated and the albumose precipitated by alcohol. This precipitate was redissolved in water, dialyzed until no chlorine reaction could be obtained with silver nitrate, the solution concentrated on the water bath to a syrup and finally precipitated with alcohol, washed with alcohol and ether and then dried at 106° C. *in vacuo* until of constant weight. In the last dialysis, there was no separation whatever of heteroalbumose, hence the protoalbumose is to be considered as quite pure. The composition of the substance is shown in the accompanying table. The ash contained no sulphate.

ANALYSIS OF A. PROTOALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure m.m.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
I	0·4428	0·2699	6·77	0·8062	49·65	....	....	.....	....	.....	....	.....	....
II	0·2665	0·1607	6·70	0·4862	49·75	....	....	.....	....	.....	....	.....	....
III	0·4825	.....	....	.....	....	63·0	15·2	753·4	15·40	.....	....	.....	....
IV	0·4038	.....	....	.....	....	52·1	14·3	758·2	15·36	.....	....	.....	....
V	0·5392	.....	....	.....	....	....	....	.....	....	0·0673	1·71	.....	....
VI	0·5516	.....	....	.....	....	....	....	.....	....	0·0733	1·80	.....	....
VII	0·6856	.....	....	.....	....	....	....	.....	....	.....	....	0·0172	2·50

Percentage composition of the ash-free substance.

							Average.
C	50·92	51·03	....	....	....	....	*50·98*
H	6·94	6·87	....	....	....	....	*6·90*
N	....	....	15·79	15·75	....	....	*15·77*
S	....	....	....	....	1·75	1·86	*1·81*
O	....	....	....	....	....	....	*24·54*
							100·00

The protoalbumose was readily soluble in water and, unlike the protoalbumose from fibrin, dissolved to a perfectly clear solution with a neutral or very faintly alkaline reaction. The aqueous solution was rendered somewhat turbid by the addition of a little acetic acid, the turbidity disappearing, however, on the addition of an excess of acid. The aqueous solution, strongly acidified with acetic acid, was precipitated by the addition of potassium ferrocyanide; the precipitate, however, dissolved on heating the mixture, reappearing as the solution became cool.

An aqueous solution of the albumose, acidified with acetic acid to such an extent that the first turbidity was re-dissolved, was not rendered at all turbid by the addition of a little sodium chloride; the addition of more salt, however, gave a very strong turbidity which disappeared entirely on warming, reappearing on cooling. As with the protoalbumose from fibrin, it is possible to add such a quantity of sodium chloride as to induce a very heavy precipitate, yet have it wholly disappear on boiling the mixture, separating out again, however, as the solution becomes cool. Finally the addition of a larger amount of sodium chloride gave a precipitate in the acidified solution, which was not at all affected by even boiling.

An aqueous solution of protoalbumose, when treated drop by drop with concentrated nitric acid, was rendered noticeably turbid at the point of contact, the turbidity disappearing as the mixture was shaken. On adding just the right proportion of nitric acid, a point was reached where the solution showed a permanent turbidity, which disappeared on the application of a little heat, returning as the solution cooled. A slight excess of nitric acid produced even in the cold, a very distinct reddish yellow coloration of the fluid, the turbidity disappearing. By adding crystals of salt to the acid solution, a precipitate was again formed, which disappeared on the application of heat, and reappeared as the solution cooled.

By saturating an aqueous solution of protoalbumose with salt, a heavy precipitate was formed, but in the filtrate more albumose was always found on the addition of a little acetic acid. In fact, each time protoalbumose was precipitated by sodium chloride in substance there was always a loss; a certain proportion of the substance remaining in the filtrate, precipitable only by the addition of a little acetic acid. Protoalbumose heated with acid, or treated in the cold with dilute alkalies was not apparently converted into acid albumin or alkali-albuminate-like bodies, for on neutralization, no precipitation whatever occurred. Heated with potassium hydroxide

and plumbic acetate, there was a decided blackening of the fluid. The protoalbumose likewise gave the characteristic reddish violet color with potassium hydroxide and cupric sulphate. Cupric sulphate alone, added to an aqueous solution of protoalbumose, gave a heavy greenish colored precipitate, not very soluble in excess of the copper salt. Mercuric chloride and lead acetate also precipitated the albumose.

In its reactions, therefore, the protoalbumose formed from egg-albumin does not differ, essentially at least, from fibrin protoalbumose.

A. Deuteroalbumose.

This body was obtained from the first salt-saturated fluid, by the addition of a little acetic acid (sp. gr. 1042) also saturated with salt. As Kühne and Chittenden have already pointed out, all of the protoalbumose is not precipitated by saturation of a neutral fluid with sodium chloride. Hence, it is to be expected that the deuteroalbumose solution would contain some protoalbumose, which latter would be likewise precipitated by the salt-saturated acetic acid. We endeavored to make a separation, however, by rejecting altogether the first precipitate produced by the addition of a little acetic acid, and then to obtain the deuteroalbumose fairly free from the former, by the subsequent addition of more acetic acid. The final precipitate so obtained, was dissolved in a small amount of water and then dialyzed for several days. The solution, in which was noticed a small deposit of heteroalbumose, was concentrated and finally precipitated by alcohol. The precipitated deuteroalbumose was then redissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed in running water for many days, after which the solution was concentrated to a syrup, the albumose precipitated with alcohol and finally, after washing with ether, dried at 106° C. *in vacuo* until of constant weight. The composition of the product is shown in the accompanying table. The ash was composed mainly of ferric oxide and calcium phosphate; it contained no sulphate.

The pure white powder, after being dried at 106° C, was found readily soluble in water. The solution was not rendered at all turbid by saturation with sodium chloride, but the substance was more or less completely precipitated by the addition of a little acetic acid to the salt-saturated fluid. Nitric acid added to an aqueous solution of the substance gave no precipitate whatever, but colored the solution decidedly yellow even in the cold. A little sodium chloride added to

ANALYSIS OF A. DEUTEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure m.m.					
I	0·5091	0·3168	6·91	0 9596	51·40	....	....	....	....	.....	....	.....	....
II	0·4387	0·2725	6·90	0·8310	51·65	....	....	....	....	.....	....	.....	....
III	0·5222	.....	....	.....	....	69·5	15·8	753·5	15·66	.....	....	.....	....
IV	0·6229	.....	....	.....	....	80·5	14·0	761·6	15·47	.....	....	.....	....
V	0·4525	.....	....	.....	....	....	....	....	....	0·0566	1·71	.....	....
VI	0.5961	.....	....	.....	....	....	....	....	....	0·0805	1·85	.....	....
VII	0·6743	.....	....	.....	....	....	....	....	....	.....	....	0·0078	1·15
VIII	0·5807	.....	....	.....	....	....	....	....	....	.....	....	0·0059	1·01

Percentage composition of the ash-free substance.

							Average.
C	51·96	52·19	-----	-----	-----	-----	*52·07*
H	6·98	6·97	-----	-----	-----	-----	*6·97*
N	-----	-----	15·83	15·64	-----	-----	*15·73*
S	-----	-----	-----	-----	1·74	1·87	*1·80*
O	-----	-----	-----	-----	-----	-----	*23·43*
							100·00

the nitric acid solution gave a decided turbidity, which disappeared on warming the solution and reappeared on cooling.

The addition of acetic acid to an aqueous solution of the albumose gave no precipitate whatever, nor was any change to be observed on heating the fluid; neutralization, at least, caused no precipitation. The addition of a little sodium chloride solution to a solution of deuteroalbumose acidified slightly with acetic acid gave no precipitate whatever, but as with deuteroalbumose from fibrin, the application of a little heat induced a slight turbidity, which disappeared on raising the temperature still higher. Again, on the further addition of sodium chloride, a heavier precipitate was produced which disappeared completely on heating the solution and reappeared on cooling; and lastly, by adding more sodium chloride, a precipitate was obtained which was permanent even on heating the mixture to boiling. In these, as in nearly all other respects, the deuteroalbumose showed itself the same in nature as the deuteroalbumose from fibrin, and the reactions given for that body can well be applied here. In one reaction only was there any very noticeable difference; viz: in the reaction with cupric sulphate. Deuteroalbumose from egg-albumin gave only a slight precipitate with cupric sulphate, even on the addition of a minimum amount of the copper salt.* With acetic acid and potassium ferrocyanide, the reaction was much the same as with protoalbumose. Boiling with sodium hydroxide and lead acetate gave a decided blackening of the fluid, from the presence of sulphur.

A. *Heteroalbumose.*

The greater portion of the heteroalbumose was obtained by the dialysis of the 5 and 10 per cent. sodium chloride solutions of the first salt precipitate, viz: in the purification of protoalbumose. Some, too, was also found in the dialysis of the precipitated deuteroalbumose. In both cases, the albumose was left as a more or less gummy precipitate, closely adherent to the parchment of the dialyser, separating out as the sodium chloride left the solution. The product was purified by solution in 5 per cent. sodium chloride, re-precipitation by the addition of salt in substance, re-solution in 5 per cent. sodium chloride and separation by dialysis, continued until all chlorine was

* This fact simply shows the greater purity of this preparation of deuteroalbumose or rather its freedom from protoalbumose, for as Dr. Neumeister has recently shown, perfectly pure deuteroalbumose gives no precipitate whatever with cupric sulphate. Later, we were able to prepare deuteroalbumose entirely free from protoalbumose.

ANALYSIS OF A. HETEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
I	0·4441	0·2720	6·80	0·8324	51·11	----	----	----	----	------	----	------	----
II	0·5365	0·3312	6·85	1·0047	51·07	----	----	----	----	------	----	------	----
III	0·3226	-----	----	------	----	42·1	15·6	754·1	15·39	------	----	------	----
IV	0·3255	-----	----	------	----	41·6	14·5	757·7	15·20	------	----	------	----
V	0·6451	-----	----	------	----	----	----	----	----	0·0751	1·59	------	----
VI	0·1772	-----	----	------	----	----	----	----	----	------	----	0·0033	1·86

Percentage composition of the ash-free substance.

						Average.
C	52·08	52·03	----	----	----	*52·06*
H	6·93	6·98	----	----	----	*6·95*
N	----	----	15·67	15·48	----	*15·55*
S	----	----	----	----	1·63	*1·63*
O	----	----	----	----	----	*23·81*
						100·00

removed from the solution. Like heteroalbumose from fibrin, this product each time it was re-dissolved in dilute sodium chloride, left a residue soluble only in dilute acids; presumably dysalbumose.

After being washed with water, alcohol and ether, the product was dried at 106° C. *in vacuo* and then analyzed with the results shown in the accompanying table.

The ash consisted mainly of calcium phosphate and a little ferric oxide, but did not contain any sulphate. The reactions of the body were found to be almost identical with those described as characteristic of heteroalbumose from fibrin.* Suspended in water or dissolved in 5–10 per cent. sodium chloride solution and then heated to boiling, the heteroalbumose was changed into a body, *coagulated heteroalbumose*, insoluble in sodium chloride but slowly soluble in 0·2 per cent. hydrochloric acid, from which it was precipitated by neutralization, apparently reconverted again, in part, into soluble heteroalbumose and in part into a body resembling dysalbumose. Thus on neutralizing the acid solution, a decided precipitate was obtained which was in part soluble in 5 per cent. sodium chloride (heteroalbumose), while the residue insoluble in the salt solution was soluble in dilute acids and in dilute alkalies (dysalbumose). In the filtrate from the neutralization precipitate, acetic acid showed the presence of still more heteroalbumose, which could be separated from the solution by dialysis.

Further, the heteroalbumose was found to be soluble in dilute acids, alkalies and alkaline carbonates, and from the solutions thus formed, it was reprecipitated by neutralization, but never completely; the amount remaining in solution being dependent naturally on the amount of neutral salt contained in the fluid.

Nitric acid precipitated the albumose, the extent of the precipitation depending on the amount of sodium chloride present. Acetic acid likewise gave a precipitate, soluble in excess of the acid. With acetic acid and potassium ferrocyanide, a precipitate was formed also soluble in excess of acid. With cupric sulphate, on the contrary, a heavy precipitate was obtained in a sodium chloride solution of the albumose, insoluble in excess of the copper salt. The substance gave the so-called biuret reaction with cupric sulphate and potassium hydroxide quite plainly, and also gave evidence of the presence of sulphur on boiling with potassium hydroxide and plumbic acetate.

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* See Zeitschrift für Biologie, Band xx, p. 32–36.

ANALYSIS OF B. PROTOALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the ash. gram.	S of Ash. % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
I	0·3540	0·1980	6·21	0·5784	44·55	----	----	------	----	----	----	------	------	------	----	-----
II	0·4965	0·2709	6·06	0·8088	44·42	----	----	------	----	----	----	------	------	------	----	-----
III	0·3794	------	----	------	----	45·5	17·8	755·2	14·05	----	----	------	------	------	----	-----
IV	0·3771	------	----	------	----	45·1	18·1	762·8	14·13	----	----	------	------	------	----	-----
V	0·6098	------	----	------	----	----	----	------	----	0·0773	12·67	------	------	------	----	-----
V*b*	0·6098	------	----	------	----	----	----	------	----	----	----	0·1018	2·29	------	----	-----
VI	0·7674	------	----	------	----	----	----	------	----	----	----	-----	------	0·2100	3·75	1·46

Percentage composition of the ash-free substance.

						Average.
C	51·00	50·88	----	----	----	*50·94*
H	7·11	6·96	----	----	----	*7·03*
N	----	----	16·09	16·18	----	*16·13*
S	----	----	----	----	2·01	*2·01*
O	----	----	----	----	----	*23·89*
						100·00

Digestion of albumin B.

340 grams of the dry albumin, non-coagulated, were soaked in 2 litres of 0·4 per cent. hydrochloric acid for 24 hours, then warmed up to 45° C. and 1 litre of the purified pepsin-hydrochloric acid added, also warmed at the same temperature. The mixture was kept at 45° C. for 16 hours, then neutralized and filtered. The filtrate, containing the albumose bodies formed from the non-coagulated albumin, was then treated as already described under Albumin A.

B. Protoalbumose.

The protoalbumose isolated from this digestion, was purified in much the same manner as A protoalbumose, and did not differ from it in its reactions, except that with water it did not dissolve to quite so clear a solution; in fact its solution in water resembled more closely the aqueous solutions of protoalbumose from fibrin. During its final purification, it was dialyzed in running water until no chlorine reaction could be obtained with silver nitrate. In spite of this fact, however, the preparation contained a large percentage of ash, consisting mainly of calcium sulphate, ferric oxide and a little calcium phosphate.

The accompanying table shows the composition of the substance after drying at 106° C., *in vacuo*, until of constant weight.

In the purification of this protoalbumose, the substance was reprecipitated three times by saturating the aqueous solution of the precipitate with sodium chloride. By this treatment, as already stated, there is considerable loss, inasmuch as the precipitation of protoalbumose with salt in this manner is never complete, considerable remaining each time in the salt-saturated fluid. By adding a very little acetic acid, however, the protoalbumose is completely precipitated from the salt-saturated solution. The filtrates therefore, from the second and third precipitations of protoalbumose with salt alone were united, and the albumose remaining in them precipitated by the addition of a little acetic acid, saturated with sodium chloride. Our object was to see whether the protoalbumose which had at one time been precipitated by salt alone and then had finally become soluble in the salt-saturated fluid, differed at all in composition or in reaction from the protoalbumose still insoluble in the salt solution.

The albumose separated in this manner was purified by being dissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed for several days. The fluid was then con-

ANALYSIS OF B. PROTOALBUMOSE.

Precipitated from NaCl solution, by CH_3–COOH.

No.	Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the ash. gram.	S of Ash. % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
I	0·5802	0·3495	6·69	1·0523	49·46	....	....	.....	....	....	....	.....	.....	.....	....	.....
II	0·3114	.....	....	.....	....	40·8	19·0	765·2	15·49	....	....	.....	.....	.....	....	.....
III	0·4942	.....	....	.....	....	....	....	.....	....	0·0142	2·87	.....	.....	.....	....	.....
III*b*	0·4942	.....	....	.....	....	....	....	.....	....	....	....	0·0091	0·25	.....	....	.....
IV	0·5084	.....	....	.....	....	....	....	.....	....	....	....	.....	.....	0·0800	2·16	1·91

Percentage composition of the ash-free substance.

				Average.
C	50·91	....	....	*50·91*
H	6·89	....	....	*6·89*
N	....	15·94	....	*15·94*
S	....	....	1·98	*1·98*
O	....	....	....	*24·28*
				100·00

centrated to a syrup and the albumose precipitated by alcohol. This precipitate was again dissolved in water, the solution made exactly neutral and again dialyzed.

After suitable concentration, the albumose was again precipitated by alcohol, washed with alcohol and ether and finally dried at 106° C. *in vacuo.*

The results of the analysis are seen in the accompanying table.

The ash in this preparation is seen to be much smaller than in the protoalbumose precipitated by salt alone. In other respects the two analyses are closely comparable, particularly the carbon and sulphur.

The reactions were in almost every case the same as with the preceding preparation, excepting perhaps a somewhat greater solubility.

B. *Deuteroalbumose.*

This body was separated and purified in exactly the same manner as in the preceding digestion. The analysis of the product is shown in the accompanying table. The ash contained some calcium sulphate and a little ferric oxide. The reactions of the body were the same as those of A deuteroalbumose.

From this digestion, more or less heteroalbumose was separated but no analysis was made of the product, as the amount was rather small for the necessary purification.

Digestion of Albumin C.

In the digestion of this sample of coagulated albumin, a much more vigorous pepsin-hydrochloric acid was employed than in the preceding digestions. The freshly coagulated albumin was placed in 3 litres of 0·4 per cent. hydrochloric acid and brought to a temperature of 45° C., then 400 c. c. of a pepsin solution, made from a pure glycerin extract of pepsin, were added and the mixture kept at 45° C. for 24 hours. The fluid was then neutralized, filtered and the clear filtrate saturated with sodium chloride. The albumose bodies were then separated and purified according to the methods already described.

The several bodies showed the same reactions as observed in the preceding preparations.

Protoalbumose and deuteroalbumose were analyzed. The results are shown in the accompanying tables. The ash of the deuteroalbumose contained no sulphate, but was composed almost entirely of ferric oxide.

ANALYSIS OF B. DEUTEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
						c. c.	T. °C.	Pressure m.m.								
I	0.3821	0·2359	6·86	0·7065	50·42	....	....	.....	....	....	....	.....	.....	.....	.....	.....
II	0·2411	0·1480	6·82	0·4453	50·37	....	....	.....	....	....	....	.....	.....	.....	.....	.....
III	0·4945	.....	....	.....	....	65·2	18·6	760·1	15·50	....	....	.....	.....	.....	....	.....
IV	0·5393	.....	....	.....	....	71·5	19·4	760·2	15.55	....	....	.....	.....	.....	....	....
V	0·5424	.....	....	.....	....	....	....	.....	....	0·0085	1·56	.....	.....	.....	....	.....
Vb	0·5424	.....	....	.....	....	....	....	.....	....	....	....	0·0055	0·09	.....	....	....
VI	0·5292	.....	...	.....	....	....	...	.....	....	....	....	.....	.....	0·0820	2·12	2·03
VII	0·3578	.....	....	.....	....	....	....	.....	....	....	....	.....	.....	0·0550	2·11	2·02

Percentage composition of the ash-free substance.

							Average.
C	51·21	51·16	....	....	....	....	*51·19*
H	6·96	6·92	....	....	....	....	*6·94*
N	....	....	15·75	15·80	....	....	*15·77*
S	....	....	....	....	2·03	2·02	*2·02*
O	....	....	....	....	....	....	*24·08*
							100·00

Dysalbumose.

This form of albumose was found in all three digestions, but the amount was much smaller than noticed in the fibrin digestions. The substance was obtained as an insoluble residue, after extracting the first sodium chloride precipitate successively with 10 and 5 per cent. salt solutions and with water. It was then dissolved in 0·2 per cent. hydrochloric acid, and after filtration precipitated by neutralization. After precipitation in this manner, a portion of the substance was found soluble in sodium chloride and on dialysis of the solution, separated in much the same manner as heteroalbumose. The portion still insoluble in salt solution was then washed thoroughly with water and lastly with alcohol and ether. Not enough of the albumose was separated from any one digestion for analysis, but by uniting the products from all three, sufficient was obtained for the following analytical data:

I. 0·2537 gram substance gave 0·1500 gram H_2O=6·57 per cent. H and 0·4550 gram CO_2=48·92 per cent. C.

II. 0·3700 gram substance gave 46·3 c. c. N at 13·8° C. and 761·3^{mm} pressure=14·96 per cent. N.

III. 0·1354 gram substance gave 0·0069 gram ash=5·09 per cent.

The ash-free substance therefore contained 51·52 per cent. C, 6·92 per cent. H, 15·79 per cent. N. The ash was composed wholly of ferric oxide.

The peculiar behavior of dysalbumose after solution in either dilute acids or sodium carbonate and neutralization, shows plainly that the substance is simply heteroalbumose rendered insoluble by action of the sodium chloride. Dysalbumose, wholly insoluble in sodium chloride, is readily dissolved by sodium carbonate of 1 per cent., and on neutralization of the alkaline fluid is in great part precipitated. The substance however, is now soluble in sodium chloride and has evidently been reconverted into heteroalbumose. It is very apparent, however, from our results that heteroalbumose from egg-albumin is not so readily converted into dysalbumose by the action of sodium chloride, as heteroalbumose from fibrin. In all three of our experiments, the amount found was very small.

Further, it would seem as if heteroalbumose from albumin was somewhat more resistant to the action of alcohol and ether than heteroalbumose from fibrin. Still the former did become quite rapidly insoluble in sodium chloride after standing under alcohol

ANALYSIS OF C. PROTOALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
						c.c.	T. °C.	Pressure m.m.								
I	0·3618	0·2087	6·41	0·6097	45·95	----	----	------	----	----	----	------	------	------	----	-----
II	0·4489	0·2547	6·30	0·7582	46·05	----	----	------	----	----	----	------	------	------	----	-----
III	0·3283	------	----	------	----	40·9	17·6	748·1	14·47	----	----	------	------	------	----	-----
IV	0·3028	------	----	------	----	----	----	------	----	0·0321	10·60	------	------	------	----	-----
IV*b*	0·3028	------	----	------	----	----	----	------	----	----	----	0·0500	2·26	------	----	-----
V	0·3962	------	----	------	----	----	----	------	----	----	----	------	------	0·1100	3·81	1·55

Percentage composition of the ash-free substance.

					Average.
C	51·39	51.48	----	----	*51·44*
H	7·16	7·05	----	----	*7·10*
N	----	---	16·18	----	*16·18*
S	----	----	----	2·00	*2·00*
O	----	----	----	----	*23·28*
					100·00

ANALYSIS OF C. DEUTEROALBUMOSE,

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure m.m.					
I	0·4262	0·2682	6·99	0·7978	51·05	....	....	.....	....	.....	....	.....	....
II	0·3394	0·2104	6·88	0·6326	50·83	....	....	.....	....	.....	....	.....	....
III	0·4465	.....	....	.....	....	59·9	18·7	760·1	15·77	.....	....	.....	....
IV	0·3451	.....	....	.....	....	46·8	20·4	756·6	15·77	.. ...	....	.....	....
V	0·4508	.....	....	.....	....	....	....	... ..	....	0·0680	2·07	.....	....
VI	0·4715	.....	....	.....	....	....	....	.....	....	0·0716	2·08	... ..	....
VII	0·4769	.....	....	.....	....	....	....	.....	....	.....	....	0·0061	1·28

Percentage composition of the ash-free substance.

							Average.
C	51·70	51·49	....	....	....	....	*51·60*
H	7·08	6·97	....	....	....	....	*7·02*
N	....	....	15·98	15·98	....	....	*15·98*
S	....	....	....	....	2·08	2·09	*2·08*
O	....	....	....	....	....	....	*23·32*
							100·00

for a short time. The reactions of dysalbumose, aside from its behavior towards sodium chloride, were found to be much the same as those of heteroalbumose.

Relation of the albumoses to albumin.

In composition, the albumoses from albumin are seen to differ from each other somewhat more than the albumoses from fibrin; collectively, however, there is less difference in composition between the albumose bodies and the albumin from which they are formed, than noticed in the case of the albumose bodies from fibrin.* In the latter, however, there is no guarantee that the fibrin employed in the experiments had the actual composition assigned to pure blood-fibrin. The fibrin-albumoses collectively contained about 50·6 per cent. of carbon and 17·1 per cent. of nitrogen, while Hammarsten found fibrin itself to contain 52·6 per cent. of carbon and 16·9 per cent. of nitrogen. In our experiments, on the other hand, we have for comparison the composition of the albumin actually used in the experiments, and in the accompanying table the differences in composition of the various products are plainly to be seen.

Examining these in detail, we see that all of the products show a somewhat smaller content of carbon than albumin itself. With nitrogen, however, there is a very close agreement throughout, and with sulphur likewise. In the case of the fibrin-albumoses it was considered that the diminished percentage of carbon indicated plainly that the albumoses were hydration products, and that they were formed from fibrin by simple hydrolytic action. The results obtained with the globuloses did not appear to confirm this view, but in this case it must be remembered that the digestion of globulin by gastric juice may be quite a different process from albumin digestion. With albumin, however, the results, although less pronounced, also indicate hydrolytic action and that the products formed are hydration products.

The following table shows the extent of these differences, and also shows the close agreement in composition between proto- and deutero-albumose and the so-called soluble and insoluble hemialbumose from egg-albumin, isolated and analyzed by Kühne and Chittenden at the commencement of their study of these bodies.*

* See Zeitschrift für Biologie, Band xix, p. 174.

	Protoalbumose.				Deuteroalbumose.			Hetero-albumose.	Dys-albumose.	Albumin.
	A	B*	B†	C	A	B	C	A	A, B & C	Average of A, B & C
C	50·98	50·94	50·91	51·44	52·07	51·19	51·60	52·06	51·52	52·33
H	6·90	7·03	6·89	7·10	6·97	6·94	7·02	6·95	6·92	6·98
N	15·77	16·13	15·94	16·18	15·73	15·77	15·98	15·55	15·79	15·85
S	1·81	2·01	1·98	2·00	1·80	2·02	2·08	1·63	....	1·82
O	24·54	23·89	24·28	23·28	23·43	24·08	23·32	23·81	....	23·02
Ash	2·50	12·67	2·87	10·60	1·08	1·56	1·28	1.86	5.09	0·55

* Sodium chloride precipitate.

† Acetic acid precipitate.

	Fibrin products.			Egg-albumin products.				
	Proto-albumose.†	Deutero-albumose.†	Fibrin.‡	Soluble Hemi-albumose.*	Insoluble Hemi-albumose.*	Proto-albumose.§	Deutero-albumose.§	Egg-albumin.
C	50·77	50·65	52·68	50·89	51·04	51·07	51·62	52·33
H	6·78	6·83	6·88	6·81	6·89	6·98	6·97	6·98
N	17·14	17·17	16·91	15·98	15·79	16·00	15·82	15·85
S	1·08	0·97	1·10	....	....	1·95	1·96	1·82
O	24·23	24·38	22·48	....	....	24·00	23·63	23·02

Deuteroalbumose is seen to contain 0·7 per cent. less carbon than egg-albumin, while protoalbumose contains fully 1·25 per cent. less. The nitrogen in the two compounds is in accord with the content of carbon and the composition of the two products certainly suggests hydrolytic action.

The lower content of carbon in the albumose bodies has been explained by some writers on the ground that the precipitants used, principally acetic acid, would tend to form an acid compound, and that even when dried and ready for analysis, the compound would still contain some acid; which fact would be sufficient to account for the low content of carbon found. It is to be noticed, however, in these results that the deuteroalbumose, not only on an average but in each individual case, contains more carbon than protoalbumose, which was precipitated by sodium chloride alone without the addition of any acetic acid whatever. Further, comparing the two preparations of protoalbumose B, one of which was precipitated by salt alone and the other by acetic acid, it is seen that the percentage of carbon is the same in both. Unquestionably proto- and deuteroalbumose both do combine with acids, but after neutralization and long continued dialysis the albumose body certainly exists in a free state, uncombined with either acid or alkali.

The greater portion of this work was completed before we had any knowledge of Dr. Neumeister's work on the complete separation of protoalbumose from deuteroalbumose.‖ It was, therefore, too late for

* See Kühne and Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskörper. Zeitschrift für Biologie, Band xix, p. 174.

† Average of all the products analyzed. Zeitschrift für Biologie, Band xx, p. 40.

‡ According to Hammarsten. § Average of all products.

‖ Zur Kenntniss der Albumosen. Zeitschrift für Biologie, Band xxiii, p. 381.

us to take advantage of his method of separation. Our preparations of deuteroalbumose unquestionably were not wholly free from protoalbumose, but that they did not contain much of this body is evidenced by the small precipitate obtained with cupric sulphate; for, as Dr. Neumeister has recently shown, deuteroalbumose entirely free from protoalbumose gives no precipitate whatever with cupric sulphate.

Casein and its Primary Cleavage Products. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Following out the general plan of procedure indicated some time ago with other albuminous bodies,* we have endeavored to prepare and study the primary products formed in the digestion of pure casein with pepsin-hydrochloric acid. Assuming that casein in its conversion into peptone by artificial gastric juice, passes through certain intermediate stages, in which bodies akin to the albumose bodies are formed, we have applied the methods of separation used so successfully in the past and have been able to isolate a class of bodies bearing the same relationship to casein that the albumoses do to albumin. For this class of bodies we propose the name of *caseoses.*

In studying these substances and particularly their composition, we deemed it essential to be certain of the purity and composition of the casein to be digested; particularly in view of the recent controversy between Hammarsten† and A. Danilewsky as to the single nature of this albuminous body. Assuming, as claimed by Danilewsky,‡ that casein is a mixture of two albuminous bodies and that the numerous analyses recently made by Hammarsten of various preparations of casein are incorrect, particularly in the percentage of sulphur, made it incumbent on us to obtain some data on these points confirmatory of one or the other view, before advancing to a study of the products formed by the digestion of casein.

Of the various methods used for the preparation of pure casein, that depending on repeated precipitation by acids and re-solution in alkalies has been the most in vogue; for although theoretical objections might be advanced as to the possibility of change in the nature of the substance under the influence of acids and alkalies, the results obtained have in some respects, at least, been very satisfactory. Alex. Schmidt, in conjunction with Kapeller,§ showed plainly that dialysis of milk and then precipitation of the casein by acetic acid, while it gave fairly good results, was not sufficient in itself to

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* See the preceding articles on globuloses and albumoses.

† See Zeitschrift für physiologische Chemie. Band vii, p. 227. Zur Frage, ob das casein ein einheitlicher stoff sei. Also same volume, p. 427.

‡ See Zeitschrift für physiologische chemie, Band vii, p. 433.

§ Beitrag zur Kenntniss der Milch, Jahresbericht für Thierchemie, 1874, p. 154.

wholly remove the inorganic salts, and thus recourse was had to repeated precipitation by acids, after solution in dilute alkalies. By this method, Hammarsten* came to the conclusion that milk contains but two albuminous bodies, viz: casein and lacto-albumin, and the same investigator has repeatedly made use of this method for the preparation of pure casein. Lundberg† has plainly shown the noticeable resistance of casein to the action of acids, and Hammarsten has indicated the possibility of using acetic acid, if necessary, in place of the stronger hydrochloric acid. Millon and Commaille, however, have claimed that in the precipitation of casein with either acid, the precipitate does not consist of free casein, but is a compound of casein with the acid used.

This erroneous view, Hammarsten shows depends simply on the great difficulty of washing the precipitated casein completely, and he suggests that it is perhaps impossible to prepare large quantities of casein absolutely pure. For the preparation of the substance, however, Hammarsten recommends acetic acid in preference to hydrochloric acid and final drying of the compound at a temperature of 110° C.

Danilewsky and Radenhausen,‡ however, prefer to use hydrochloric acid in the preparation of pure casein, and for this purpose they use skimmed milk, diluted with 4–5 volumes of water, to which the dilute acid is added little by little, until a good precipitate is obtained. After filtration and repeated washing with distilled water, the casein is rubbed fine, then dissolved in water to which a little ammonia has been added, the fluid filtered and the clear filtrate again precipitated by the addition of a little dilute hydrochloric acid. Casein, so prepared, after being washed with distilled water, reacts acid to test papers and shows the usual reactions of this body; but if when freshly precipitated, the substance is boiled with perfectly neutral 50 per cent. alcohol and filtered hot, according to Danilewsky and Radenhausen, the casein is separated into two bodies, one of which is partially soluble in hot alcohol and separates out on cooling, while the other is insoluble. The soluble portion is termed caseoprotalbin, the insoluble portion caseoalbumin. The former, it is stated, gives

* Zur Kenntniss des Caseins und der Wirkung des Labfermentes, Jahresbericht für Thierchemie, 1877, p. 158.

† Kleinere Beiträge zur Kenntniss des Caseins, Jahresbericht für Thierchemie, 1876, p. 11.

‡ Untersuchungen über die Eiweisstoffe der Milch, Jahresbericht für Thierchemie, 1880, p. 186.

no sulphur reaction when boiled with 2 per cent. sodium hydroxide, but contains 1·13 per cent. of sulphur, while caseoalbumin is stated to contain 1·23 per cent. of sulphur. Hence, according to these investigators, casein is a mixture of two bodies, one of which is rich in sulphur, while the other contains a somewhat smaller amount. The objection which these investigators make to the use of acetic acid in the preparation of casein, is that from a sodium acetate solution, casein itself is not precipitated, or only in part, but that the precipitate consists mainly of the protalbin body. Further, Danilewsky and Radenhausen claim that caseoalbumin dissolved in 1 per cent. sodium hydroxide and allowed to stand for 24 hours at the temperature of the room, is changed almost completely into caseoprotalbin, with loss of sulphur and calcium phosphate. In a similar manner, protalbin dissolved in lime water, with addition of alcohol and phosphoric acid, can be changed into caseoalbumin.

Hammarsten,* however, takes exception to these views and points out that the peculiar behavior of Danilewsky's casein towards boiling 50 per cent. alcohol, depends in part upon its content of calcium phosphate, the presence of which impurity depends upon the use of hydrochloric acid in the precipitation of the casein, which acid does not favor the removal of the salt as well as acetic acid. Using acetic acid as the precipitant and then employing a casein three times so precipitated, and which analysis showed to be almost entirely free from calcium phosphate, Hammarsten, by treatment with boiling alcohol, was unable to obtain anything more than a trace of substance corresponding to caseoalbumin. Further, casein is unquestionably changed by boiling with alcohol, as Hammarsten clearly shows; in fact it is well known that heating an albuminous body in water is liable to change its nature, at least its solubility, and there is no reason why treatment with 50 per cent. alcohol should not lead to a like result. Again, Hammarsten points out clearly another inconsistency in the reasoning of Danilewsky and Radenhausen in connection with the so-called conversion of caseoprotalbin into caseoalbumin. The former body is stated to be poorer in sulphur than the latter, and yet we are told that the protalbin body can be converted into caseoalbumin by simple solution in lime water and addition of phosphoric acid, with or without alcohol. Yet how it is possible by this method of treatment to convert a body with a small

* Zur Frage, ob das Casein ein einheitlicher Stoff sei. Zeitschrift für physiologische chemie, Band vii, p. 227.

content of sulphur into a body richer in sulphur, is hard to see. Much more plausible is it, as suggested by Hammarsten, that in these two bodies we have to deal with the same substance, in the one case united with calcium phosphate, and in the other uncombined with this salt; or in other words that the so-called protalbin body in the absence of calcium phosphate is soluble in boiling 50 per cent. alcohol, while in the presence of that salt it is insoluble.

This view being correct, and Hammarsten's observations would tend to show that it is, it is obvious that the caseoprotalbin of Danilewsky is simply a portion of the casein, which, owing to lack of a sufficient amount of calcium phosphate, passes into solution on being boiled with dilute alcohol; while caseoalbumin, on the other hand, is likewise a portion of the casein, *insoluble* on account of the presence of calcium phosphate; changed, however, more or less by action of the boiling alcohol. Further, the reason why casein precipitated several times by acetic acid does not contain as much calcium phosphate as when precipitated by hydrochloric acid, and thus reacts differently with alcohol, depends on the far greater insolubility of freshly precipitated casein in excess of acetic acid than in hydrochloric acid. In the precipitation of casein with hydrochloric acid only the slightest excess of acid can be added, on account of the ready solubility of the precipitate in this dilute acid. With acetic acid, however, a moderate excess can be added without solution of the precipitate, and thus in the latter case, a larger proportion of mineral salts are removed at each re-precipitation.

Danilewsky and Radenhausen have further called attention to the fact that casein precipitated with hydrochloric acid yields a larger amount of alkaline sulphide than when precipitated by acetic acid. This statement, Hammarsten has several times been able to verify, but the latter investigator seeks an explanation for this fact in the occasional presence of a second albuminous body, richer in sulphur, presumably serum-globulin, precipitable like casein by acids. Serum-globulin too, is readily soluble in excess of acid, even more so than casein, and hence by the acetic acid method of precipitation, which allows a far greater excess of acid, the casein would be much less liable to contamination by this hypothetical globulin than by the hydrochloric acid method. In this connection it may be well to notice that Musso and Menozzi* claim the presence in milk of a peculiar albuminous body containing 53·74 per cent. C, 15·52 per

* Studien über das Eiweiss der Milch. Jahresbericht für Thierchemie, 1878, p. 139.

cent. N and 1·55 per cent. S, for which they claim a position midway between serum-albumin and casein. It can be partially precipitated from fresh milk at ordinary temperatures by the addition of acetic acid. Further, Lebelien* has proved the presence in milk of a globulin-like body, lacto-globulin, which can be precipitated by saturating the fluid remaining after removal of the casein with sodium chloride, with magnesium sulphate. The substance appears to be identical with paraglobulin, and thus this fact, just discovered, would seem to confirm Hammarsten's theory as to the cause of the greater content of sulphur sometimes noticed in casein precipitated by hydrochloric acid.

Accepting then, Hammarsten's views as correct, it is obvious that casein precipitated by acetic acid, if not a single body, must be composed of two bodies, more or less alike and both precipitable by dilute acids. In attempting to settle this point definitely, Hammarsten has sought by analysis of a large number of preparations made under different conditions, to obtain data as to the exact composition of casein variously prepared. Naturally in this connection, considerable attention was paid to the content of sulphur, since, Danilewsky and Radenhausen's views being correct, variations in the content of sulphur would naturally be expected. If casein is a mixture of equal parts of caseoprotalbin and caseoalbumin with 1·13 per cent. and 1·23 per cent. of sulphur, respectively, then casein itself would naturally contain 1·18 per cent. of sulphur; an amount somewhat higher than has been found heretofore.

Recently, Dainlewsky† has modified his views somewhat, and now considers casein, as before, to be a mixture of two distinct bodies, but of nucleoalbumin with nucleoprotalbin instead of caseoalbumin and caseoprotalbin. As the reactions of these two bodies are apparently much the same as those given as characteristic of the caseobodies, this change of view appears to be mainly a change of name. Danilewsky still claims the correctness of the high content of sulphur in casein and assumes that the variation in the results obtained by different workers is due simply to difference in the methods of determination, and that unquestionably pure casein contains over 1·0 per cent. of sulphur.

The content of sulphur in casein as determined 30–40 years ago by

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* Beitrag zur Kenntniss der Eiweisskörper der Kuhmilch. Jahresbericht für Thierchemie, 1885, p. 184.

† See Zeitschrift für physiologische chemie, Band vii, p. 433.

Lehmann, Rühling, Völckel and others,* varies from 0·85 per cent. to 1·10 per cent. Ritthausen,† from several analyses of the copper compound of casein, found a content of sulphur equivalent to 0·80–1·12 per cent. in the free casein. Schwarzenbach,‡ by a study of the platinum cyanide compound of casein, ascribed to casein itself a content of 0·19–1·10 per cent. of sulphur. Hammarsten, however, found by analysis of eight distinct preparations of casein, some of which had been reprecipitated even ten times with acetic acid, a content of sulphur ranging from 0·619 per cent. to 0·775 per cent.§ Later, Hammarsten‖ analyzed four other preparations of casein and each by six distinct methods. Omitting two or three results, which were altogether too low on account of inaccuracies in the method, Hammarsten found in these different preparations of casein, as a result of twenty-nine distinct determinations by five different methods, 0·798 per cent. as maximum, 0·726 per cent. as minimum, or 0·758 per cent. as the average, content of sulphur. Taking, however, the results obtained by what Hammarsten considers as the more correct methods the average content of sulphur is raised to 0·77–0·78 per cent. In no case did Hammarsten obtain results in any way confirmatory of Danilewsky's views. Hammarsten further made a large number of phosphorus determinations, and these as well as the results obtained for carbon and nitrogen showed too little variation to warrant the idea of a mixture of two bodies of unlike composition. While therefore, Hammarsten's results would seem to point conclusively to the unit-like nature of casein, we have, however, made quite a number of different preparations of the substance, both from fresh milk and from skimmed milk, with the idea of obtaining confirmatory data, with which to make direct comparisons between the composition of casein and its primary cleavage products.

Preparation and composition of Casein.

The casein was precipitated in some cases by acetic and in others by hydrochloric acid. In both cases the acid used was very dilute,

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* See Gmelin-Krauts' Handbuch der Organische Chemie, Band iv, Abtheilung, iii, 1870, p. 2254.

† H. Ritthausen und R. Pott, Untersuchungen über Verbindungen der Eiweisskörper mit Kupferoxyd. Journal für prakt. Chemie, 1873, Band vii, p. 361.

‡ Annalen der Chem. u. Pharmacie, Band cxxxiii, p. 185.

§ Zeitschrift für physiologische Chemie, Band vii, p. 259.

‖ Ueber den Gehalt des caseins an Schwefel und über die Bestimmung des Schwefels in Proteinsubstanzen. Zeitschrift für physiologische chemie, Band ix, p. 273.

the hydrochloric acid being 0·2 per cent. In dissolving the casein for re-precipitation, a very dilute solution of ammonium hydroxide was employed; in fact so dilute as to consist of hardly more than water with a trace of ammonia. We used ammonia, in preference to sodium or potassium hydroxide, as this alkali would seem less liable to induce any alteration in the content of sulphur. Further, in dissolving the casein in ammonia, the solution at no time became more than very faintly, if at all alkaline; usually being hardly more than neutral to test papers.

The general method of procedure was to dilute fresh cow's milk with about four volumes of water (skimmed milk diluted considerably less) and then to precipitate the casein with either hydrochloric or acetic acids, adding the precipitant cautiously, until complete precipitation was obtained. The precipitate was then washed as completely as possible with large quantities of water, both by decantation, trituration with water in a mortar and on a cloth filter. The casein was then dissolved in the ammonia water, filtered through paper and reprecipitated, each time being thoroughly washed with water. In the portion used for analysis, the final precipitate was further washed with alcohol and ether and lastly soaked in a mixture of alcohol and ether for the more complete removal of any fat. The preparations were then dried in the air and lastly on a water bath at a gentle heat. When dry, they were powdered and extracted with boiling ether in a fat extractor for several hours, to insure complete freedom from fat. Ultimately, the products for analysis were dried at 105° C. *in vacuo* until of constant weight.

In all, seven preparations of casein were made for analysis, as follows:

No. I. From fresh milk, precipitated twice with hydrochloric acid.
" II. From fresh milk, precipitated twice with acetic acid.
" III. From skimmed milk, precipitated three times with acetic acid.
" IV. A portion of No. III, precipitated a fourth time with acetic acid.
" V. From skimmed milk, precipitated three times with hydrochloric acid.
" VI. A portion of No. V, precipitated a fourth time with hydrochloric acid.
" VII. From skimmed milk, precipitated four times with hydrochloric acid.

The methods of analysis were essentially the same as those employed by Kühne and Chittenden in the analysis of the various albumose bodies. Carbon and hydrogen were determined by combus-

tion with oxygen in an open tube, the gases passing over a long layer of granular oxide of copper at a bright red heat, a layer of lead chromate at a dull red heat and a roll of freshly reduced metallic copper. Nitrogen was determined as nitrogen gas by combustion with oxide of copper, the gases passing over a long anterior layer of heated oxide, a short layer of metallic copper and a final layer of oxide of copper. The tube was exhausted with a Sprengel pump before and after the combustion and the nitrogen was collected in a Schiffs' azotometer, provided with a jacket tube for rapid cooling of the gas to a constant temperature. In the determination of sulphur and phosphorus, the substance was fused with a mixture of potassium hydroxide and potassium nitrate (10 grams of the former and 1·5 grams of the latter) in a silver crucible, according to the method designated by Hammarsten * as 1*a*. In order to economize time, a single fusion was made to serve for both a sulphur and phosphorus determination; in other words, a sufficient amount of casein (usually 1·2 grams) was fused with potassium hydroxide and nitrate, the fused mass dissolved in water, the solution made up to a known volume and then one-half, representing one-half of the original substance, was used for the sulphur, the other half for the phosphorus determination. Both the alkali and the nitrate were free from sulphur and phosphorus; at least to such an extent that in a blank experiment, the resultant solutions gave no precipitate whatever, with either barium chloride or with molybdic solution. The oxidations were made at as low a temperature as possible, except towards the end when the temperature was raised, and occasionally a little more nitrate added, to facilitate complete oxidation.

As the percentage of sulphur was quite an important point, we took particular pains to have the final acid fluid entirely free from nitrate and nitrite, as well as from any excess of hydrochloric acid, so as to avoid as much as possible any solvent action on the barium sulphate. For the determination of sulphur, therefore, the alkaline solution of the fused mass was acidified distinctly with hydrochloric acid and the acid solution evaporated to perfect dryness on the water-bath. In this way the objectionable nitrate and nitrite were removed. The residue was then moistened with hydrochloric acid, taken up in water, and the solution allowed to stand until any chloride of silver present, had settled out. The fluid was then filtered and precipitated as usual with barium chloride. For phos-

* Zeitschrift für physiologische Chemie, Band ix, p. 289.

ANALYSIS OF CASEIN No. I.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
I	0·4104	0·2605	7·05	0·7935	52·72	....	....	....	....	.....	....	.....	....	....	....
II	0·3499	0·2214	7·03	0·6762	52·70	....	....	....	....	.....	....	.....	....	....	....
III	0·4850	....	....	....	....	65·6	20·2	765·2	15·72	.....	....	.....	....	....	....
IV	0·3751	....	....	....	....	50·5	20·0	763·0	15·80	.....	....	.....	....	....	....
V	0·6227	....	....	....	....	....	....	....	....	0·0874	0·82	.....	....	....	....
VI	0·6227	....	....	....	....	....	....	....	....	.....	....	0·0189	0·85	....	....
VII	0·4469	....	....	....	....	....	...	....	....	.....	....	.....	....	0·0043	0·96
VIII	0·4756	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0046	0·96

Percentage composition of the ash-free substance.

							Average.
C	53·23	53·21	....	....	....	....	*53·22*
H	7·11	7·09	....	....	....	....	*7·10*
N	....	....	15·89	15·97	....	....	*15·93*
S	....	....	....	....	0·83	....	*0·83*
P	....	....	....	....	....	0·86	*0·86*
O	....	....	...	....	....	....	*22·06*
							100·00

ANALYSIS OF CASEIN No. II.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $HOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Asb. %
I	0·3685	0·2300	6·93	0·7137	52·81	----	----	----	----	------	----	------	----	----	----
II	0·4938	0·3120	7·02	0·9590	52·96	----	----	----	----	------	----	------	----	----	----
III	0·3653	----	----	----	----	49·2	21·2	758·2	15·66	------	----	------	----	----	----
IV	0·3542	----	----	----	----	47·5	20·6	756·5	15·77	------	----	------	----	----	----
V	0·5986	----	----	----	----	----	----	----	----	0·0819	0·73	------	----	----	----
VI	0·7403	----	----	----	----	----	----	----	----	0·0417	0·77	------	---	----	----
VII	0·5986	----	----	----	----	----	----	----	----	------	----	0·0187	0·87	----	----
VIII	0·7403	----	----	----	----	----	----	----	----	------	----	0·0284	0·88	----	----
IX	0·5536	----	----	----	----	----	----	----	----	------	----	------	----	0·0035	0·63
X	0·5324	----	----	----	----	----	----	----	----	------	----	------	----	0·0030	0·56

Percentage composition of the ash-free substance.

									Average.
C	53·12	53·27	----	----	----	----	----	----	*53·19*
H	6·97	7·06	----	----	----	----	----	----	*7·01*
N	----	----	15·75	15·86	----	----	----	----	*15·80*
S	----	----	----	----	0·74	0·78	----	----	*0·76*
P	----	----	----	----	----	----	0·88	0·89	*0·88*
O	----	----	----	----	----	----	----	----	*22·36*
									100·00

ANALYSIS OF CASEIN No. III.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
I	0·3865	0·2451	7·04	0·7453	52·58	....	....	....	....	.....	....	.....	....	....	....
II	0·3805	0·2375	6·94	0·7335	52·57	....	....	....	....	.....	....	.....	....	....	....
III	0·3566	....	....	....	....	48·3	21·0	757·4	15·74	.....	....	.....	....	....	....
IV	0·3686	....	....	....	....	51·1	21.0	757,1	15·88	.....	....	.....	....	....	....
V	0·5466	....	....	....	....	....	....	....	....	0·0350	0·88	.....	....	....	....
VI	0·5466	....	....	....	....	....	....	....	....	.....	....	0·0172	0·88	....	....
VII	0·4835	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0065	1·34
VIII	0·4290	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0061	1·42

Percentage composition of the ash-free substance.

							Average.
C	53·31	53·30	....	....	....	....	*53·30*
H	7·14	7·04	....	....	....	....	*7·09*
N	....	....	15·95	16·04	....	....	*15·99*
S	....	....	....	....	0·89	....	*0·89*
P	....	....	....	....	....	0·89	*0·89*
O	....	....	....	....	....	....	*21·84*
							100·00

ANALYSIS OF CASEIN No. IV.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
I	0·3276	0·2080	7·02	0·6371	53·03	....	....	....	....	.....	....	.....	....	....	....
II	0·4500	0·2807	6·93	0·8720	52·84	....	....	....	....	.....	....	.....	....	....	....
III	0·3642	....	....	....	....	48·5	20·4	764·6	15·66	.....	....	.....	....	....	....
IV	0·4003	....	....	....	....	54·1	20·6	764·6	15·87	.....	....	.....	....	....	....
V	0·6124	....	....	....	....	....	....	....	....	0·0850	0·78	.....	....	....	....
VI	0·6006	....	....	....	....	....	....	....	....	0·0868	0·84	.....	....	....	....
VII	0·6124	....	....	....	....	....	....	....	....	.....	....	0·0189	0·86	....	....
VIII	0·6006	....	....	....	....	....	....	....	....	.....	....	0·0181	0·87	....	....
IX	0·3887	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0084	0·87
X	0·3802	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0029	0·76

Percentage composition of the ash-free substance.

									Average.
C	53·49	53·30	....	....	....	....	....	....	*53·39*
H	7·11	7·02	....	....	....	....	....	....	*7·06*
N	....	....	15·77	15·98	....	....	....	....	*15·87*
S	....	....	....	....	0·79	0·85	....	....	*0·82*
P	....	....	....	....	....	....	0·87	0·88	*0·87*
O	....	....	....	....	....	....	....	....	*21·99*
									100·00

ANALYSIS OF CASEIN No. V.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	Ash found. gram.	Ash. %
I	0·5970	0·3813	7·09	1·1575	52·87	----	----	----	----	------	----	------	----	----	----
II	0·6132	----	----	1·1934	52·91	----	----	----	----	------	----	------	----	----	----
III	0·3084	0·1950	7·02	0·5960	52·70	----	----	----	----	------	----	------	----	----	----
IV	0·3663	----	----	----	----	48·8	18·4	756·9	15·57	------	----	------	----	----	----
V	0·3828	----	----	----	----	51·4	19·4	756·7	15·67	------	----	------	----	----	----
VI	0·8350	----	----	----	----	----	----	----	----	0·0449	0·74	------	----	----	----
VII	0·8350	----	----	----	----	----	----	----	----	------	----	0·0249	0·83	----	----
VIII	0·5279	----	----	----	----	----	----	----	----	------	----	------	----	0·0045	0·85
IX	0·5316	----	----	----	----	----	----	----	----	------	----	------	----	0·0046	0·86

	Percentage composition of the ash-free substance.							Average.
C	53·31	53·35	53·14	----	----	---	----	*53·27*
H	7·15	----	7·08	----	----	----	----	*7·11*
N	----	----	----	15·70	15·80	----	----	*15·75*
S	----	----	----	----	----	0·75	----	*0·75*
P	----	----	----	----	----	----	0·84	*0·84*
O	----	----	----	----	----	----	----	*22·28*
								100·00

Analysis of Casein No. VI.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	Ash found. gram.	Ash. %
I	0·3774	0·2357	6·94	0·7302	52·76	....	....	....	....	.....	....	.....	....	....	....
II	0·3643	0·2294	6·99	0·7040	52·69	....	....	....	....	.....	....	.....	....	....	....
III	0·3520	....	....	....	....	47·8	19·6	760·3	15·92	.....	....	.....	....	....	....
IV	0·3640	....	....	....	....	49·7	20·4	760·3	15·96	.....	....	.....	....	....	....
V	0·6219	....	....	....	....	....	....	....	....	0·0365	0·80	.....	....	....	....
VI	0·6220	....	....	....	....	....	....	....	....	0·0420	0·92	.....	....	....	....
VII	0·6219	....	....	....	....	....	....	....	....	.....	....	0·0195	0·87	....	....
VIII	0·6220	....	....	....	....	....	....	....	....	.....	....	0·0193	0·85	....	....
IX	0·4436	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0046	1·03
X	0·4398	....	....	....	....	....	....	....	....	.....	....	.....	....	0·0045	1·02

Percentage composition of the ash-free substance.

									Average.
C	53·29	53·21	....	....	....	....	....	....	*53·25*
H	7·00	7·05	....	....	....	....	....	....	*7·02*
N	....	....	16·07	16·10	....	....	....	....	*16·08*
S	....	....	....	....	0·81	0·93	....	....	*0·87*
P	....	....	....	....	....	....	0·88	0·86	*0·87*
O	....	....	....	....	....	....	....	....	*21·91*
									100·00

ANALYSIS OF CASEIN NO. VII.

No.	Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$ gram.	P %	Ash found. gram.	Ash. %
I	0·5031	0·3161	6·98	0·9760	52·90	----	----	------	----	------	----	------	---	----	----
II	0·4063	0·2589	7·08	0·7870	52·82	----	----	------	----	------	----	------	---	----	----
III	0·4755	------	----	------	----	64·1	20·0	757·3	15·72	------	----	------	---	----	----
IV	0·3980	------	----	------	----	53·6	19·2	760·5	15·82	------	----	------	---	----	----
V	0·6782	------	----	------	----	----	----	------	----	0·0429	0·86	------	---	----	----
VI	0·6782	------	----	------	----	----	----	------	----	------	----	0·0210	0·86	----	----
VII	0·4059	------	----	------	----	----	----	------	----	------	----	------	---	0·0056	1·37
VIII	0·4118	------	----	------	----	----	----	------	----	------	----	------	---	0·0052	1·26

Percentage composition of the ash-free substance.

							Average.
C	53·58	53·49	-----	-----	-----	-----	*53·53*
H	7·07	7·14	-----	-----	-----	-----	*7·10*
N	-----	-----	15·92	16·01	-----	-----	*15·96*
S	-----	-----	-----	-----	0·88	-----	*0·88*
P	-----	-----	-----	-----	-----	0·88	*0·88*
O	-----	-----	-----	-----	-----	-----	*21·65*
							100·00

phorus, the alkaline fluid was acidified with nitric acid, evaporated to dryness, the residue dissolved in a little water acidified with nitric acid, filtered, and the phosphoric acid precipitated in the usual manner with ammonium molybdate. After standing 24 hours at 40° C. this precipitate was filtered off, dissolved in ammonium hydroxide and the phosphoric acid re-precipitated as ammonio magnesium phosphate and ultimately weighed as magnesium pyrophosphate. The accompanying tables show the results of the analyses of the different samples of casein.

Comparing now the average composition of these different preparations of casein, it is to be seen that they all show a very close agreement throughout. Thus the percentage of phosphorus in the seven preparations varies only from 0·84 to 0·89, sulphur from 0·75 to 0·89, nitrogen from 15·75 to 16·08, hydrogen from 7·01 to 7·11 and carbon from 53·19 to 53·53; or leaving out one preparation which for some reason showed a high content of carbon, from 53·19 to 53·39 per cent. The results therefore show a constancy in composition fully as marked as observed by Hammarsten and thus tend to confirm the latter in the view that casein is a single body of definite composition.

Comparing our results collectively, with those obtained by Hammarsten (see table showing average composition), we find a fairly close agreement throughout, although minor differences are to be observed. First, all of our preparations show a content of carbon somewhat higher than found by Hammarsten. The latter investigator found the carbon in his preparations to vary from 52·78 to 53·09 per cent., while in all of our preparations, the content of carbon calculated to the ash-free substance is above 53 per cent. The possibility of our preparations still containing some fat was rendered improbable by the thorough treatment with ether which they had received, and further by the fact that the nitrogen in our preparations was also somewhat higher than found by Hammarsten. One of the preparations, however, with a high content of carbon, was extracted again for several hours with boiling ether, but on analysis the content of carbon was found unchanged. The content of phosphorus agrees exactly with Hammarsten's results, while the sulphur is, on an average, 0·1 per cent. higher. There is nothing in the content of sulphur, therefore, to even suggest confirmation of Danilewsky's views. The amount of ash in our preparations was somewhat larger than found by Hammarsten and further, there is no especial connection to be seen between the content of ash and the precipitant used; the

TABLE SHOWING THE AVERAGE COMPOSITION OF THE CASEINS.

	No. I. twice precipitated with HCl.	No. II. twice precipitated with CH_3-COOH.	No. III. 3 times precipitated with CH_3-COOH.	No. IV. 4 times precipitated with CH_3-COOH.	No. V. 3 times precipitated with HCl.	No. VI. 4 times precipitated with HCl.	No. VII. 4 times precipitated with HCl.	Average of Nos. I–VII.	Average of Hammarsten's results.*	According to Ritthausen †
C	53·22	53·19	53·30	53·39	53·27	53·25	53·53	53·30	52·96	54·22
H	7·10	7·01	7·09	7·06	7·11	7·02	7·10	7·07	7·05	7·17
N	15·98	15·80	15·99	15·87	15·75	16·08	15·96	15·91	15·65	15·49
S	0·88	0·76	0·89	0·82	0·75	0·87	0·88	0·82	0·72	0·91
P	0·86	0·88	0·89	0·87	0·84	0·87	0·88	0·87	0·85	....
O	22·06	22·36	21·84	21·99	22·28	21·91	21·65	22·08	22·78	....
ash	0·96	0·59	1·38	0·81	0·85	1·02	1·31	0·98		

* See Zeitschrift für physiologische chemie, Band vii, p. 269.

† Calculated by Ritthausen from analysis of the copper compound of casein, average of three results. See Jahresbericht für Thierchemie, 1873, p. 28.

amount in the acetic acid precipitate being fully as large as in the casein precipitated by hydrochloric acid.

Compared with Ritthausen's results (see table showing average composition), obtained by analysis of the copper compound of casein, the percentage of carbon comes very much too high. It is questionable, however, how close a comparison should be drawn between indirect results obtained by analysis of a metallic compound of casein and those obtained by analysis of casein itself.

In conclusion then, we must affirm that our results accord closely with those of Hammarsten's, while the two together make it very improbable that in casein we have to do with a substance composed of two bodies of unlike composition.

Digestion of Casein and Formation of Caseoses.

In the digestion of casein with pepsin-hydrochloric acid, the casein was prepared by precipitation and reprecipitation with acetic acid, and rendered as pure as possible by thorough washing with water. While still moist it was placed in 0·4 per cent. hydrochloric acid, as preliminary to its treatment with pepsin. Pure pepsin solution, free from peptone and albumose, was prepared from the mucous membrane of pig's stomachs by the method already described.*

Digestion A.

1300 grams of moist casein in 4 litres of 0·4 per cent. hydrochloric acid were brought to a temperature of 45° C. and 600 c.c. of pure pepsin solution added. The mixture was kept at a temperature of 45° C. throughout the digestion. The casein began almost immediately to swell up and in less than an hour the entire mixture was converted into a semi-solid, jelly-like mass. Thereupon, one litre more of 0·4 per cent. hydrochloric acid was added, together with a little more pepsin solution. At the end of three hours, the mixture was quite fluid, but contained considerable gelatinous matter in suspension. Neutralization of a filtered portion, produced no precipitate whatever. The addition of crystals of sodium chloride gave a heavy white precipitate and the filtrate from this precipitate gave a further precipitate on the addition of acetic acid. At the end of four hours, the entire mixture was made neutral with sodium hydroxide and then filtered through paper. The undigested residue, when dry, amounted to about 30 or 40 grams. This residue of so-called casein dyspeptone,

* See the preceding article on egg-albumin and albumoses.

which appeared in every digestion in greater or less quantity, was apparently wholly insoluble in fresh portions of gastric juice and was similar in its reactions to the like-body previously described by Lubavin.* When fresh, it appeared as a more or less jelly-like mass, much like starch paste. It was readily soluble in dilute alkalies and precipitated by neutralization, but insoluble in excess of acid. It was precipitated from its solution in dilute sodium hydroxide by addition of salt in substance. This body we did not attempt to study further, but hope to do so later.

The filtrate from the undigested residue, when cold, was not perfectly clear but became so on the application of a gentle heat. On boiling the solution, a very slight precipitate was formed. The addition of acids, hydrochloric, nitric or acetic, either concentrated or dilute, caused a heavy white precipitate, not wholly soluble in excess of acid, even of concentrated hydrochloric. The precipitate was likewise more or less permanent when warmed or even boiled with the acid. In the filtrate from the precipitate produced by acetic acid, the addition of potassium ferrocyanide gave no precipitate. The precipitate produced by acids was readily soluble in dilute alkalies. On addition of nitric acid, of any strength, and the application of heat even to boiling, the mixture turned first rose color then reddish, and as the boiling was continued the color deepened and finally became brownish red. The change from rose color to brown also takes place in the cold. With concentrated nitric acid the color is nearer the yellow of the xanthoprotein reaction, but still shows plainly the brown or reddish tinge.

For separation of the individual caseoses, the entire solution without being concentrated, was saturated with sodium chloride, by which an exceedingly heavy precipitate, more or less curdy, was obtained, which was finally filtered off and washed with saturated salt solution. The washing was made more thorough by grinding the mass with the salt solution in a mortar. This precipitate, by analogy, would naturally be composed mainly of a body corresponding to protoalbumose with possibly something corresponding to heteroalbumose. The precipitate was washed thoroughly with saturated salt solution, dissolved in water, filtered, and again precipitated by saturation of the fluid with sodium chloride. All of the substance, however, was not reprecipitated; quite a little remained in the salt-

* Ueber die Künstliche Pepsin-Verdauung des caseins, etc., Hoppe-Seyler's Med. Chemische Untersuchungen, p 467.

saturated fluid and was thrown down as a white curdy precipitate by the addition of a little acetic acid, evidently some protocaseose not precipitated by salt alone. The main precipitate of protocaseose, etc., twice precipitated by salt, was treated with 3 litres of 10 per cent. salt solution, the residue with 3 litres of 5 per cent. salt solution and the residue still remaining, with 3 litres of water. In this manner, all of the proto and heterocaseose was dissolved, leaving a small residue wholly insoluble in dilute salt solutions and in water; presumably dyscaseose. The latter, however, was in exceedingly small quantity. It was dissolved in 0·2 per cent. hydrochloric acid and reprecipitated by neutralization of the solution with sodium carbonate.

A. *Protocaseose.*

The 5 and 10 per cent. salt solutions of protocaseose together with the aqueous solution, were united and the mixture saturated with sodium chloride. Here, as before, all of the protocaseose was not precipitated; a portion remained in the filtrate and was precipitated only on the addition of a little acetic acid. The main portion of the protocaseose precipitated for the third time with salt in substance, was dissolved in water, the solution filtered and divided into two parts. One part was thymolized and dialyzed in running water until all chlorine was removed from the solution (Protocaseose A 1). The other part was again saturated with salt, the precipitate washed with saturated salt solution, then dissolved in water and like the former dialyzed until all chlorine was removed from the solution (Protocaseose A 2). In protocaseose 2, there was more evidence of the presence of a body resembling heteroalbumose than in No. 1. Thus in No. 2, quite a little gummy substance separated from the solution on dialysis, but the amount even here was not large. When the dialysis was finished, both solutions were perfectly neutral to test papers and in both cases the protocaseose was separated from the clear fluid by evaporation and precipitation with alcohol. For analysis, both products were washed thoroughly with alcohol and ether and finally dried at 105° C. *in vacuo.*

Their composition is shown in the accompanying tables.

As already stated, every time protocaseose was dissolved in water and reprecipitated by saturating the solution with sodium chloride, a certain amount of the substance remained in solution, precipitable only on addition of a little acetic acid. Protocaseose precipitated from the salt-saturated solution in this manner by acetic acid,

ANALYSIS OF PROTOCASEOSE A 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N. %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·4722	0·2851	6·70	0·8519	49·20	....	....	....	....	.....	....	.....	....
II	0·3360	.....	....	0·6080	49·34	....	....	....	....	.....	....	.....	....
III	0·3500	.....	....	.....	....	43·7	20·4	766·2	14·71	.....	....	.....	....
IV	0·3850	.....	....	.....	....	48·3	19·7	766·2	14·81	.....	....	.....	....
V	0·6060	.....	....	.....	....	....	....	....	....	0·0380	0·86	.....	....
VI	0·5025	.....	...	.....	....	....	....	....	....	.....	....	0·0312	6·20

Percentage composition of the ash-free substance.

						Average.
C	52·44	52·57	....	....	....	*52·50*
H	7·15	....	....	....	....	*7·15*
N	....	....	15·68	15·78	....	*15·73*
S	....	....	....	....	0·96	*0·96*
O	....	....	....	....	....	*23·66*
						100·00

ANALYSIS OF PROTOCASEOSE A 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the ash. gram.	S of Ash. % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
I	0·3729	0·2284	6·80	0·6895	50·42	----	----	------	----	----	----	------	------	------	----	-----
II	0·3934	0·2366	6·69	0·7265	50·38	----	----	------	----	----	----	------	------	------	----	-----
III	0·3636	------	----	------	----	45·8	20·1	766·1	14·85	----	----	------	------	------	----	-----
IV	0·3562	------	----	------	----	44·9	20·2	764·2	14·82	----	----	------	------	------	----	-----
V	0·4923	------	----	------	----	----	----	------	----	0·0316	6·42	------	------	------	----	-----
V*b*	0·4923	------	----	------	----	----	----	------	----	----	----	0·0231	0·64	------	----	-----
VI	0·5925	------	----	------	----	----	----	------	----	----	----	-----	------	0·0659	1·52	0·88

Percentage composition of the ash-free substance.

						Average.
C	53·87	53·83	----	----	----	*53·85*
H	7·27	7·16	----	----	----	*7·21*
N	----	----	15·86	15·83	----	*15·84*
S	----	----	----	-----	0·98	*0·98*
O	----	----	----	----	----	*22·12*
						100·00

was found to be quite different in its nature from protoalbumose or protoglobulose. The two latter undoubtedly combine with acetic acid, when precipitated from a salt-saturated fluid; the compound, however, is readily and completely soluble in water. With protocaseose on the other hand, acetic acid produces a precipitate, not only insoluble in the salt-saturated fluid, but also more or less insoluble in water containing a little acid. It is easily soluble in dilute alkali and alkali carbonate, and is not precipitated by neutralization with hydrochloric or acetic acid. Addition of acid, however, beyond neutralization immediately causes precipitation of the caseose.

Protocaseose once precipitated with salt and which on the second precipitation, failed to separate from the salt-saturated fluid, was precipitated by a little acetic acid, washed somewhat with water, dissolved in very dilute sodium carbonate solution, neutralized and then dialyzed in running water for nearly a week. The solution was then perfectly neutral to test papers and was likewise perfectly clear. It was evaporated to a syrup and the caseose precipitated by alcohol. After being washed with alcohol and ether, it was dried at 105° C. *in vacuo* until of constant weight, and then analyzed with the following results:

Protocaseose A 3.

I. 0·3703 gram substance gave 0·2160 gram $H_2O = 6·48$ per cent. H and 0·6500 gram $CO_2 = 47·86$ per cent. C.

II. 0·4200 gram gave 50·9 c. c. N at 21·2° C. and 765·0^{mm} pressure = 14·21 per cent. N.

III. 0·7053 gram fused with KOH and KNO_3 gave 0·0418 gram $BaSO_4$ = 0·81 per cent. S.

IV. 0·4076 gram gave 0·0386 gram ash = 9·47 per cent.

The ash-free substance therefore contained

52·59% C. 7·17% H. 15·70% N. 0·90% S.

Like all of the caseose bodies, this contained a large percentage of ash in spite of its long continued dialysis. The ash was mainly calcium phosphate with some oxide of iron, obtained in part doubtless from the salt used in precipitation. It is not difficult to see how protocaseose, precipitated by salt alone, should take up and retain semi-mechanically considerable inorganic matter. But in the present case, where the great mass of the albuminous substance has been precipitated by the salt added, it seems somewhat surprising that the caseose should separate from the clear fluid in the presence of

considerable acetic acid with such a large percentage of adherent mineral matter, unless the latter is chemically combined with the albuminous substance. Oft-repeated and long continued dialysis appears to have but little influence in diminishing the amount of this impurity. Our experience has taught us that where the caseoses have once been brought in contact with lime salts, reprecipitation and other methods of purification avail but little.

But very little heterocaseose was found in this digestion, not as much as was found in some of the others, later on. Still, during the first dialysis of the protocaseose, some little heterocaseose separated from the fluid, as the last traces of salt dialyzed out.

A. *Deuterocaseose.*

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate of protocaseose, by acetic acid. As a study of the reactions of protocaseose had shown plainly that this body is never completely precipitated by salt alone, a little acetic acid was added to the salt-saturated filtrate, and the precipitate, presumably a mixture of proto and deuterocaseose, thrown away. The remaining deuterocaseose was then precipitated by adding about 200 c.c. of a salt-saturated acetic acid (30 per cent. acetic acid) to the fluid. The total volume of the mixture was nearly 16 litres. In this manner an abundance of a finely divided precipitate was obtained, which at first, seemed insoluble in water and in dilute sodium chloride. It was readily soluble in water containing a trace of alkali and was not precipitated by neutralization. On being washed, however, with a saturated salt solution for some time, the washings were found to have dissolved considerable of the substance, which could be precipitated from the solution by strong acetic acid. Further, after being washed with salt solution, the substance remaining appeared quite noticeably soluble in water. Evidently then, this body on being washed more or less free from acid, becomes soluble in water to a certain extent, its aqueous solution then giving a strong reaction with acetic acid and potassium ferrocyanide. The great bulk of the precipitate was therefore washed with saturated salt solution until the washings were nearly free from acid; then, having become partially soluble in water, it was placed in about 2 litres of water, the solution ultimately saturated with sodium chloride and the caseose again precipitated by addition of about 20 c. c. of acetic acid. This second precipitate was washed somewhat with salt solution and finally with a large volume of water. At first, the washings gave no reaction with

ANALYSIS OF DEUTEROCASEOSE A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·4027	0·2261	6·23	0·6850	46·38	----	----	-----	----	-----	----	-----	-----
II	0·4002	0·2265	6·27	0·6787	46·24	----	----	-----	----	-----	----	-----	-----
III	0·3736	-----	----	-----	----	45·8	18·0	765·9	14·07	-----	----	-----	-----
IV	0·3550	-----	----	-----	----	42·4	18·4	766·2	14·17	-----	----	-----	-----
V	0·6024	-----	----	-----	----	----	----	-----	----	0·0294	0·67	-----	-----
VI	0·6030	-----	----	-----	----	----	----	-----	----	0·0299	0·68	-----	-----
VII	0·3489	-----	----	-----	----	----	----	-----	----	-----	----	0·0358	10·26

Percentage composition of the ash-free substance.

							Average.
C	51·66	51·52	----	----	----	----	*51·59*
H	6·96	7·00	----	----	----	----	*6·98*
N	----	----	15·68	15·78	----	----	*15·73*
S	----	----	----	----	0·74	0·75	*0·75*
O	----	----	----	----	----	----	*25·03*
							100·00

acetic acid and potassium ferrocyanide, but later on, these reagents gave a heavy precipitate, showing plainly that the substance was dissolving. The entire precipitate was thereupon dissolved in very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed. After remaining in the dialyzers for nearly a week the fluid was removed, filtered from some heterocaseose which had separated, evaporated to a syrup on the water-bath and precipitated with alcohol. This precipitate was re-dissolved in water, the solution made exactly neutral to test papers and again dialyzed. From this solution, the caseose was finally precipitated by alcohol, after suitable concentration of the fluid, washed with alcohol and ether and dried at 105° C. *in vacuo*. The final solution, prior to precipitation by alcohol, was perfectly neutral and quite clear, showing no evidence of the presence of any heterocaseose.

The composition of the substance is shown in the accompanying table.

Digestion B.

In this digestion, 750 grams of freshly prepared casein were mixed with 4 litres of 0·4 per cent. hydrochloric acid, the mixture warmed at 45° C., and then 800 c. c. of pure pepsin solution added. The mixture was warmed at the above temperature for one hour and a half, then neutralized and filtered, and the clear filtrate saturated with sodium chloride. This precipitate, as in the preceding digestion, was washed thoroughly with saturated salt solution, then successively extracted with 10 and 5 per cent. salt solution and finally with water, leaving a small residue of dyscaseose soluble only in 0·2 per cent. hydrochloric acid. The united filtrates, containing proto and heterocaseose, were again precipitated with salt and then treated as described under protocaseose B.

B. Protocaseose.

The protocaseose formed in this digestion and twice precipitated by salt, was dissolved again in water, filtered through paper and then dialyzed until no chlorine reaction could be obtained with silver nitrate. Quite a little heterocaseose separated from the solution during dialysis, which was removed by filtration. The fluid was then concentrated, the substance precipitated by alcohol, again dissolved in water and dialyzed. This time, as no heterocaseose separated from the fluid, the solution was concentrated, precipitated

ANALYSIS OF PROTOCASEOSE B 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
I	0·3844	0·2268	6·56	0·6892	48·89	....	....	.....	....	....	....	.....	.....	.....	....	.....
II	0·4268	0·2504	6·51	0·7642	48·82	....	....	.....	....	....	....	.....	.....	.....	....	.....
III	0·4758	.....	....	.....	....	58·5	19·6	760·8	14·42	....	....	.....	.....	.....	....	.....
IV	0·4054	.....	....	.....	....	50·3	20·2	759·8	14·50	....	....	.....	.....	.....	....	....
V	0·3629	.....	....	.....	....	....	....	.....	....	0·0278	7·66	.....	.....	.....	....	.....
V*b*	0·3629	.....	....	.....	....	....	....	.....	....	....	....	0·0200	0·75	.....	....	....
VI	0·6254	.....	....	.....	....	....	....	.....	....	....	....	.....	.....	0·0696	1·52	0·77

Percentage composition of the ash-free substance.

						Average.
C	52·94	52·88	....	....	....	*52·91*
H	7·09	7·04	....	....	....	*7·06*
N	....	....	15·61	15·69	....	*15·65*
S	....	....	....	....	0·90	*0·90*
O	....	....	....	....	....	*23·48*
						100·00

ANALYSIS OF PROTOCASEOSE B 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·5084	0·2897	6·33	0·8808	47·44	....	....	.....	....	.....	....	.....	....
II	0·3520	0·2001	6·31	0·6130	47·49	....	....	.....	....	.....	....	.....	....
III	0·4521	.....	....	.....	....	56·0	19·6	763·5	14·58	.....	....	.....	....
IV	0·5010	.....	....	.....	....	62·5	20·4	762·3	14·62	.....	....	.....	....
V	0·6021	.....	....	.....	....	....	....	.....	....	0·0367	0·83	.....	....
VI	0·6010	.....	....	.....	....	....	....	.....	...	0·0353	0·81	.....	....
VII	0·3940	.....	....	.....	....	....	....	.....	....	.....	....	0·0389	9·87

Percentage composition of the ash-free substance.

							Average.
C	52·41	52·46	....	....	....	....	*52·43*
H	7·02	7·00	....	....	....	....	*7·01*
N	....	....	16·17	16·21	....	....	*16·19*
S	....	....	....	....	0·91	0·89	*0·90*
O	....	....	....	....	....	....	*23·47*
							100·00

with alcohol and the substance finally dried at 105° C. *in vacuo* (Protocaseose B 1). Its composition is shown in the accompanying table.

Such portion of the protocaseose as was not precipitated the second time by salt alone, was precipitated by a little acetic acid. At first, the precipitated substance was insoluble in water (no reaction with acetic acid and potassium ferrocyanide), but after being washed with salt solution until the acid reaction had nearly disappeared, it then dissolved quite appreciably in water, as evidenced by the reaction with acetic acid and potassium ferrocyanide. The bulk of the precipitate, after being washed, was dissolved in a little very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed until all chlorine was removed from the solution. The caseose, after concentration of the solution, was precipitated by alcohol, washed with alcohol and ether and finally dried at 105° C. *in vacuo*. The composition of the substance (Protocaseose B 2) is shown in the accompanying table.

B. Deuterocaseose.

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate, in the same manner as in the preceding digestion. Like A. deuterocaseose, this precipitate was at first insoluble in salt solution and in water, but after being washed for some time with saturated salt solution, it was found to be gradually dissolved, as shown by the rapid disappearance of the precipitate and the pronounced reaction with acetic acid and potassium ferrocyanide in the wash-fluid. Evidently the acid is easily removed from the compound by simple washing and when that has been effected, the substance becomes soluble, or else the acid compound is more insoluble in water containing a little free acid, than in water or salt solution alone. Hydrochloric acid and acetic acid seem to act alike. The substance is readily soluble in dilute sodium carbonate and is not precipitated by neutralization, but is quickly thrown down by a slight excess of hydrochloric or acetic acid. This compound was not analyzed, but was used in studying the reactions to be described later.

Digestion C.

In both of the preceding digestions, the products formed resulted from the action of an exceedingly vigorous pepsin mixture. In the

present case, the pepsin solution employed was much weaker and the pepsin-casein mixture was warmed at 45° C. for several days instead of hours.

750 grams of casein were employed and 4 litres of 0·4 per cent. hydrochloric acid, to which was added a reasonable amount of pure pepsin solution. About an hour after the addition of the latter the mass began to gelatinize, and at the end of 18 hours the whole mixture was a perfectly stiff jelly. Thereupon, 2 litres more of 0·4 per cent. acid were added together with a little pepsin. The mixture was then kept at 40–45° C. for three days longer. Quite a large residue of undigested matter remained, semi-gelatinous and soluble only in alkalies. The mixture was then neutralized and filtered. The filtrate contained considerable caseose, as evidenced by the heavy precipitate obtained on saturating a portion with sodium chloride. In the preceding digestions, the caseoses were separated directly from the dilute solutions, without previous concentration, thereby avoiding any possible change due to the action of heat. In the present case, however, the perfectly neutral solution was evaporated to a small volume and then all of the caseoses were directly precipitated by saturating the fluid with ammonium sulphate. The precipitate produced was exceedingly gummy, but was washed as thoroughly as possible by trituration with a saturated solution of ammonium sulphate. The caseoses were then dissolved in water, the solution filtered from the small residue of insoluble matter and saturated with sodium chloride. The protocaseose so separated was freed from heterocaseose, etc. by repeated precipitation and dialysis.

Carbon and hydrogen were determined in a portion of the dried substance (Protocaseose C 1) with the following results:

I. 0·5107 gram substance gave 0·3101 gram H_2O=6·74 per cent. H and 0·9386 gram CO_2=50·11 per cent C.

II. 0·4038 gram gave 0·0194 gram ash = 4·80 per cent.

The ash-free substance would, therefore, contain 52·64 per cent. of carbon and 7·08 per cent. of hydrogen.

In the filtrate from the first salt precipitate, the protocaseose remaining was precipitated by the addition of a little salt-saturated acetic acid. The precipitate after being washed was dissolved in a little dilute sodium carbonate, the solution neutralized and dialyzed.

As no heterocaseose separated from the solution, it was concentrated and then precipitated by alcohol. After purification by resolution, dialysis, etc., it was dried at 105° C. *in vacuo* and analyzed with the results shown in the accompanying table (Protocaseose C 2).

ANALYSIS OF PROTOCASEOSE C 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure mm.					
I	0·4080	0·2658	6·51	0·7028	46·90	----	----	----	----	------	----	------	----
II	0·4235	0·2458	6·45	0·7258	46·74	----	----	----	----	------	----	------	----
III	0·3395	------	----	------	----	41·4	19·2	759·4	14·30	------	----	------	----
IV	0·3580	------	----	------	----	43·9	20·1	766·2	14·46	------	----	------	----
V	0·6008	------	----	------	----	----	----	----	----	0·0815	0·72	------	----
VI	0·4068	------	----	------	----	----	----	----	----	------	----	0·0386	9·48
VII	0·4314	------	----	------	----	----	----	----	----	------	----	0·0399	9·24

Percentage composition of the ash-free substance.

						Average.
C	51·81	51.65	----	----	----	*51·73*
H	7·18	7·12	----	----	----	*7·15*
N	----	----	15·77	15·93	----	*15·85*
S	----	----	----	----	0·79	*0·79*
O	----	----	----	----	----	*24·48*
						100·00

Deuterocaseose was then separated from the filtrate from the acetic acid precipitate, by saturation of the fluid with ammonium sulphate, as recommended by Neumeister* for deuteroalbumose. It was then purified by dialysis, etc., and its reactions carefully studied. So far as we could see, it differed from deuterocaseose A and B in two respects only, but these points showed so marked a difference it was plainly evident that the deuterocaseose separated by ammonium sulphate was quite different from deuterocaseose A, separated by acetic acid. Thus deutero C was not precipitated at all in an aqueous solution by acetic acid, nor by acetic acid and potassium ferrocyanide, neither was its aqueous solution precipitated by cupric sulphate. The significance of these points of difference will be discussed later on. After studying the reactions, there was not enough substance remaining for analysis.

Digestion D.

In this digestion, 2 kilos of freshly prepared casein were used, together with 6 litres of 0·4 per cent. hydrochloric acid and an appropriate quantity of strong pepsin solution. The mixture was warmed at 45° C. for five hours, then neutralized and filtered from the semi-gelatinous residue.

D. *Protocaseose.*

The neutral fluid was concentrated to about 1½ litres and then filtered from the slight flocculent precipitate which had formed. Saturation of the fluid with sodium chloride gave an exceedingly heavy precipitate, somewhat more gummy than usual. The entire fluid, however, was only partially saturated with salt, with a view to see whether the precipitate produced in this manner would agree wholly with the precipitate produced on complete saturation. Thus a fractional precipitation was made, in which the first fraction represents that portion of the caseose precipitated by about two-thirds saturation of the fluid with salt. This precipitate was therefore filtered off, washed as usual, dissolved in water and re-precipitated with salt. As dyscaseose was generally found only in traces, the precipitate, after being washed, was dissolved at once in water and dialyzed until all chlorine was removed from the solution. On opening the dialyzing tubes, quite a large quantity of heterocaseose was found adherent to the sides of the paper. The clear solution of

* See Zeitschrift für Biologie, Band xxiii, p. 381.

ANALYSIS OF PROTOCASEOSE D 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %	$Mg_2P_2O_7$ from the ash. gram.	P of ash, % of substance.	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of ash, %
I	0·5211	0·3268	6·95	0·9890	51·80	--	--	----	----	-----	--	----	--	----	----	-----	--	----
II	0·4062	0·2508	6·86	0·7745	51·99	--	--	----	----	-----	--	----	--	----	----	-----	--	----
III	0·4528	----	--	----	----	59·1	18·4	762·3	15·40	-----	--	----	--	----	----	-----	--	----
IV	0·5675	----	--	----	----	48·3	18·5	762·3	15·50	-----	--	----	--	----	----	-----	--	----
V	0·6014	----	--	----	----	--	--	----	----	0·0348	0·79	----	--	----	----	-----	--	----
VI	0·6004	----	--	----	----	--	--	----	----	0·0874	0·85	----	--	----	----	-----	--	----
VII	0·4473	----	--	----	----	--	--	----	----	-----	--	0·0162	3·84	----	----	-----	--	----
VIII	0·4521	----	--	----	----	--	--	----	----	-----	--	0·0168	3·71	----	----	-----	--	----
IX	0·4049	----	--	----	----	--	--	----	----	-----	--	----	--	0·0099	0·68	-----	--	----
X	0·6014	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0149	0·69	0·01
XI	0·6004	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0147	0·68	0

Percentage composition of the ash-free substance.

							Average.
C	53·84	54·03	----	----	----	----	*53·93*
H	7·21	7·12	----	----	----	----	*7·17*
N	----	----	16·00	16·10	----	----	*16·05*
S	----	----	----	----	0·82	0·88	*0·85*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*22·00*
							100·00

ANALYSIS OF PROTOCASEOSE D 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·4196	0·2511	6·65	0·7607	49.43	----	---	-----	-----	-----	----	-----	----
II	0·3511	0·2103	6·65	0·6378	49.53	----	---	-----	-----	-----	----	-----	----
III	0·4789	------	----	------	----	60·5	18·8	762·1	14·88	-----	----	-----	----
IV	0·3626	------	----	------	----	45·7	19·2	762·1	14·88	-----	----	-----	----
V	0·6129	------	----	------	----	----	---	-----	-----	0·0414	0·92	-----	----
VI	0·6350	------	----	------	----	----	---	-----	-----	0·0482	1·04	-----	----
VII	0·4220	------	----	------	----	----	---	-----	-----	-----	----	0·0267	6·32
VIII	0·4186	------	----	------	----	----	---	-----	-----	-----	----	0·0270	6·45

Percentage composition of the ash-free substance.

							Average.
C	52·79	52·88	----	----	----	----	*52·84*
H	7·10	7·10	----	----	----	----	*7·10*
N	----	----	15·89	15·84	----	----	*15·86*
S	----	----	----	----	0·99	1.10	*1·04*
O	----	----	----	----	----	----	*23·16*
							100·00

protocaseose was concentrated, precipitated with alcohol, the precipitate dissolved in water, again precipitated with salt in substance, the precipitate dialyzed, this time without showing any heterocaseose, and finally precipitated with alcohol, washed with alcohol and ether and dried at 105° C. *in vacuo* (Protocaseose D 1). The product was analyzed with the results shown in the preceding table.

On adding more sodium chloride to the first filtrate from the above precipitate, thereby completely saturating the solution, a second precipitate of protocaseose was obtained, which was purified in the same manner as the preceding preparation. The only difference noticed while purifying the substance was that, on dialysis, nothing corresponding to heterocaseose separated from the fluid. After final washing with alcohol and ether, the substance was analyzed with the results shown in the accompanying table (Protocaseose D 2).

The difference in the results will be discussed later on.

The original salt-saturated filtrate was precipitated with a little acetic acid, and the protocaseose so precipitated dissolved in dilute sodium carbonate. The solution was then neutralized and dialyzed in running water until all chlorine was removed. The substance was then separated by precipitation with alcohol, and ultimately purified as described previously (Protocaseose D 3). The addition of a little more acetic acid to the acetic acid and salt-saturated filtrate from the above, gave a still further precipitate of caseose; presumably protocaseose with perhaps a trace of deuterocaseose, which was filtered off, washed with saturated salt solution and then freed from acid and purified in the same manner as the preceding preparation (Protocaseose D 4).

The two last products, after being dried at 105° C. *in vacuo*, were analyzed with the results shown in the following tables.

Comparing these two tables, it is seen that the difference in percentage composition of the two ash-free substances is not very great, and taking into consideration the large percentage of ash, it is probable that the two latter precipitates have approximately the same composition.

D. *Deuterocaseose.*

The filtrate, from which certainly all protocaseose had been removed by acetic acid, and in fact nearly everything precipitable by acid from the salt-saturated fluid, was treated with ammonium sulphate in substance. A gummy precipitate resulted, which naturally enclosed considerable salt, and which for purification was dis-

ANALYSIS OF PROTOCASEOSE D 3.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %
						c.c.	T. °C.	Pressure m.m.					
I	0·4072	0·2416	6·59	0·7199	48·21	----	----	----	----	----	----	----	----
II	0·4384	0·2622	6·64	0·7767	48·31	----	----	----	----	----	----	----	----
III	0·3562	----	----	----	----	44·7	18·4	766·6	14·89	----	----	----	----
IV	0·3556	----	----	----	----	45·4	18·1	765·8	15·01	----	----	----	----
V	0·4036	----	----	----	----	----	----	----	----	0·0296	7·33	----	----
VI	0·4614	----	----	----	----	----	----	----	----	----	----	0·0337	1·00
VII	0·3984	----	----	----	----	----	----	----	----	----	----	0·0279	0·96

Percentage composition of the ash-free substance.

							Average.
C	52·01	52·10	----	----	----	----	*52·05*
H	7·11	7·16	----	----	----	----	*7·13*
N	----	----	16·07	16·18	----	----	*16·12*
S	----	----	----	----	1·08	1·04	*1·06*
O	----	----	----	----	----	----	*23·64*
							100·00

ANALYSIS OF PROTOCASEOSE D 4.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·3156	0·1907	6·71	0·5816	50·25	----	----	-----	----	------	----	------	----
II	0·4575	0·2768	6·71	0·8408	50·11	----	----	-----	----	------	----	------	----
III	0·3818	-----	----	-----	----	49·6	18·6	763·1	15·84	------	----	------	----
IV	0·3627	-----	----	-----	----	46·8	18·6	766·8	15·80	------	----	------	----
V	0·6093	-----	----	-----	----	----	----	-----	----	0·0418	0·94	-----	----
VI	0·6047	-----	----	-----	----	----	----	-----	----	0·0412	0·93	------	----
VII	0·4237	-----	----	-----	----	----	----	-----	----	------	----	0·0217	5·12
VIII	0·4103	-----	----	-----	----	----	----	-----	----	------	----	0·0210	5·11

Percentage composition of the ash-free substance.

							Average.
C	52·95	52·81	----	----	----	----	*52·88*
H	7·07	7·07	----	----	----	----	*7·07*
N	----	----	16·16	16·11	----	----	*16·13*
S	----	----	----	----	0·99	0·98	*0·98*
O	----	----	----	----	----	----	*22·94*
							100·00

ANALYSIS OF DEUTEROCASEOSE D.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %	$Mg_2P_2O_7$ from the ash. gram.	P of ash, % of substance.	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of ash %
I	0·4992	0·3030	6·74	0·9047	49·42	--	--	----	----	-----	--	----	--	----	----	-----	--	----
II	0·3644	0·2200	6·71	0·6591	49·32	--	--	----	----	-----	--	----	--	----	----	-----	--	----
III	0·4112	----	--	----	----	52·8	18·5	766·1	15·23	-----	--	----	--	----	----	-----	--	----
IV	0·3860	----	--	----	----	49·7	18·0	766·9	15·30	-----	--	----	--	----	----	-----	--	----
V	0·6034	----	--	----	----	--	--	----	----	0·0486	1·10	----	--	----	----	-----	--	----
VI	0·6020	----	--	----	----	--	--	----	----	0·0500	1·14	----	--	----	----	-----	--	----
VII	0·4083	----	--	----	----	--	--	----	----	-----	--	0·0191	4·74	----	----	-----	--	----
VIII	0·4022	----	--	----	----	--	--	----	----	-----	--	0·0189	4·62	----	----	-----	--	----
VIII*b*	0·4022	----	--	----	----	--	--	----	----	-----	--	----	--	0·0097	0·67	-----	--	----
IX	0·6034	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0150	0·69	0·02
X	0·6020	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0151	0·70	0·03

Percentage composition of the ash-free substance.

							Average.
C	51·84	51·74	----	----	----	----	*51·79*
H	7·07	7·04	----	----	----	----	*7·05*
N	----	----	15·97	16·03	----	----	*16·00*
S	----	----	----	----	1·15	1·19	*1·17*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*23·99*
							100·00

solved in water and dialyzed until the greater portion of the salt was removed. The solution was then concentrated and the substance reprecipitated by saturating the neutral solution with ammonium sulphate. This precipitate, after solution in water, was then dialyzed until all of the ammonium sulphate was removed, after which the solution was concentrated and precipitated with alcohol. When dry, the substance gave by analysis the results shown in the accompanying table.

This body differs from all of the preceding preparations, in that it is not precipitated from an aqueous solution by acetic acid. Neither is it precipitated at all by the addition of salt in substance; but the addition of a little acetic acid to the salt-saturated fluid gives a heavy precipitate which, however, does not represent all of the deuterocaseose, since the filtrate gives an additional precipitate with ammonium sulphate. Apparently about one-half of the substance is precipitated by acetic acid. Further, the acetic acid precipitate in this case differs from the protocaseose precipitate with acid, in that it is readily and completely soluble in water. This body, therefore, which certainly must represent *pure* deuterocaseose, shows a close resemblance to the pure deuteroalbumose separated by Neumeister. Like the latter, it does not give any precipitate whatever with cupric sulphate nor with ferric chloride and only the faintest turbidity with acetic acid and potassium ferrocyanide.

D. *Heterocaseose.*

In each digestion, evidence was obtained at various points in the process of separation, noticeably on dialysis of the first protoalbumose precipitate, of the presence of a body insoluble in water but soluble in dilute sodium chloride solution. The quantity of the substance, however, was in most cases exceedingly small, so much so that nothing more than a few reactions could be tried with it. In the present digestion, however, the amount was somewhat larger, and sufficed for a partial analysis. The substance was obtained as a more or less gummy residue, on dialysis of protoalbumose 1. It was purified by solution in 10 per cent. sodium chloride and separation by dialysis. Like heteroalbumose, the whole of the substance was not now soluble in salt solution, for a portion had apparently been converted into a body resembling dysalbumose, insoluble in salt solution but soluble in 0·2 per cent. hydrochloric acid.

Analysis of the dried substance gave the following results :

I. 0·4011 gram substance gave 0·2463 gram H_2O=6·82 per cent. H and 0·7484 gram CO_2=50·54 per cent. C.

II. 0·4287 gram gave 53·6 c. c. N at 18·6° C. and 760·1 mm. pressure =14·70 per cent. N.

III. 0·4118 gram gave 0·0256 gram ash =6·21 per cent.

The ash-free substance would therefore contain

53·88 per cent. C, 7·27 per cent. H, 15·67 per cent. N.

Reactions of the caseoses.

Under this head little need be said. The reactions characteristic of the albumose bodies in general will apply here. Certain differences, however, have already appeared in our description of the processes incident to separation of the caseoses. Protocaseose, unlike protoalbumose, is precipitated from an *aqueous* solution by acetic acid. The precipitation, however, is not complete; saturation of the acid filtrate with sodium chloride, invariably gives an additional precipitate which is the heavier of the two. Further, long-continued washing of the acid precipitate with water or salt solution appears to partially remove the acid from the caseose body. Protocaseose is likewise precipitated from an aqueous solution by hydrochloric, nitric and sulphuric acids; the precipitate, however, is far less soluble in excess of sulphuric or nitric acid than in excess of the other two acids. In very dilute acids, protocaseose is soluble and is partially precipitated by addition of stronger acid of the same kind; thus the substance is readily soluble in 0·4 per cent. hydrochloric acid, from which solution it is precipitated by the addition of a little concentrated acid, this precipitate dissolving on the addition of more acid. Evidently then, protocaseose as fast as formed by the action of pepsin-hydrochloric acid, would dissolve in the acid gastric juice and not be mixed with the jelly-like insoluble residue. Boiled with dilute or strong acid, protocaseose is apparently not changed; at least no precipitate is obtained on neutralization of the acid fluid. The acetic acid solution of protocaseose gives a very heavy precipitate with potassium ferrocyanide. In an aqueous solution of the substance, cupric sulphate gives a heavy curd-like precipitate, while ferric chloride gives a similar precipitate readily soluble in excess of the precipitant. Like protoalbumose, protocaseose is precipitated by saturation of its aqueous solution with sodium chloride, but never completely; there always remains in the

filtrate, a portion of the substance precipitable only on addition of acetic acid.

Of the several preparations of deuterocaseose, those precipitated by ammonium sulphate are evidently the only ones perfectly pure. Further, it is evident that this substance can be obtained pure only by complete removal of all protocaseose from the solution, which implies precipitation of a large portion of the deuterocaseose also, and then precipitation of the small amount of deutero remaining, by saturation of the fluid with ammonium sulphate. D deutero, prepared in this manner, shows several very marked points of difference from protocaseose. In the first place, it is not precipitated in an *aqueous* solution by acetic acid. Further, the addition of potassium ferrocyanide to a solution acidified with acetic acid gives no precipitate whatever. Cupric sulphate and ferric chloride both fail to produce any precipitate in an aqueous solution. Pure deuterocaseose, as already mentioned, is not precipitated by saturation of its aqueous solution with sodium chloride; addition of acetic acid, however, to the salt-saturated solution gives a heavy precipitate, which represents perhaps half of the deutero, the remainder of which is precipitated only by saturation of the fluid with ammonium sulphate. It is thus evident that in the precipitation of protocaseose from a salt-saturated solution by acetic acid, more or less deuterocaseose will be likewise precipitated, the amount depending probably on the concentration of the solution and other minor circumstances. Hence, A deutero is unquestionably contaminated with some protocaseose, and on the other hand protocaseose D 3 and 4, and perhaps protocaseose C 2, without doubt contain some deuterocaseose. That *A* deutero contains some protocaseose, is evident from the fact that it gives a precipitate with cupric sulphate, and further its aqueous solution is rendered decidedly turbid by acetic acid. Moreover, the protocaseose precipitated by acetic acid, and which may contain some deutero, appears to differ in one or two respects from either proto or deuterocaseose. Thus, an aqueous solution of the purified substance is precipitated like pure protocaseose by acetic acid, but the precipitate is only partially soluble in excess of the acid and even that requires a large excess.

With nitric acid, pure deuterocaseose gives no precipitate, but on warming the solution the xanthoprotein reaction comes out strongly.

Composition of the caseoses and their relation to casein.

In studying the composition of the various caseoses we have been hampered by the large percentage of ash invariably present in all of

TABLE SHOWING RELATIVE COMPOSITION OF THE CASEOSES.

Protocaseose.	C	H	N	S	O
A 1 NaCl precipitate	52·50	7·15	15·73	0·96	23·66
A 2 " "	53·85	7·21	15·84	0·98	22·12
A 3 Acetic acid precipitate	52·59	7·17	15·70	0·90	23·64
B 1 NaCl precipitate	52·91	7·06	15·65	0·90	23·48
B 2 Acetic acid precipitate	52·43	7·01	16·19	0·90	23·47
C 1 NaCl precipitate	52·64	7·08	----	----	----
C 2 Acetic acid precipitate	51·78	7·15	15·85	0·79	24·48
D 1 NaCl precipitate	53·93	7·17	16·05	0·85	22·00
D 2 " "	52·84	7·10	15·86	1·04	23·16
D 3 Acetic acid precipitate	52·05	7·13	16·12	1·06	23·64
D 4 " " "	52·88	7·07	16·13	0·98	22·94
Deuterocaseose.					
A. Acetic acid precipitate	51·59	6·98	15·73	0·75	25·03
D. $(NH_4)_2SO_4$ "	51·79	7·05	16·00	1·17	23·99
Heterocaseose.					
D.	53·88	7·27	15·67	----	----
Casein.					
Average of Nos. I–VII	53·30	7·07	15·91	0·82	22·03

the preparations. We have already commented on the difficulty, in fact, impossibility, of removing certain inorganic salts after they have once been brought in contact with a caseose body. Repeated precipitation appears to affect the percentage of ash but little. The reason for the large percentage of ash lies in the precipitation of the caseoses from such large volumes of fluid. We thought it unwise at first, to expose the bodies to the long continued evaporation necessary for precipitation with a small amount of salt. To avoid the possible danger of change, therefore, the large volumes of fluid resulting from the several digestions were saturated directly with salt, and as this involved the use of large quantities, calcium salts and some iron as impurities in the sodium chloride, were unavoidably introduced. These, the caseoses seemed at once to catch hold of and retain, in spite of oft-repeated purification. In the digestion *D*, in which the fluid was concentrated somewhat before precipitation, the percentage of ash is seen to be somewhat smaller than in preparations from the other digestions.

In comparing the composition of the individual protocaseoses (see the accompanying table) it is seen that two of the bodies show a content of carbon somewhat higher than casein itself, while the average of all the others, with one exception, shows a content of carbon a little lower than casein. Leaving out the acetic acid precipitate C 2, the average of the remaining ten preparations of protocaseose shows the following composition for this substance:

	C	*H*	*N*	*S*	*O*
Protocaseose	52·89	7·10	15·94	0·95	23·12
Casein	53·80	7·07	15·91	0·82	22·03

Plainly, the average of our results would indicate that protocaseose does not differ essentially in composition, from the casein from which it is formed. A slightly smaller content of carbon is the only noticeable difference. To be sure the individual results show noticeable variation in the percentage of carbon, but bearing in mind the large amount of ash present in the preparations, it is evident that the average result is of more value than the results obtained in any one case. As to the lower content of carbon in so-called protocaseose C 2, it is probable that this body is composed mainly of deuterocaseose. The two caseoses being precipitated together in this digestion by ammonium sulphate and then separated afterwards from a fairly concentrated solution by saturation with salt and addition of acetic acid, renders it probable that the protocaseose was more completely precip-

itated than usual by salt alone; and further, it is probable that on addition of acetic acid to the concentrated and salt-saturated fluid, a much larger proportion of deuterocaseose was precipitated. In confirmation of this view it was noticed that the amount of deuterocaseose obtained by the later precipitation with ammonium sulphate was quite small; far smaller proportionally than obtained in D. That the body contained some protocaseose, was evident from its reaction with cupric sulphate and with acetic acid.

Pure deuterocaseose evidently contains a smaller content of carbon than protocaseose. It is equally evident that it is a body further removed from casein than protocaseose. Its general reactions show a closer relationship to peptone than to casein or the proto-body. Heterocaseose, on the other hand, judging from analysis of a single preparation, contains fully as much if not more carbon than casein itself.

Nearly all of the caseoses show a somewhat higher percentage of sulphur than casein, but probably the increase (0·1 per cent.) is due mainly to a trace of sulphate in the ash, not accounted for. Owing to the large amount of phosphate in the ash of the different preparations, phosphorus was sought for only twice. In both of these, however (protocaseose D 1 and deuterocaseose D), the phosphorus in the ash was the exact equivalent of the total phosphorus found after fusion with potassium hydroxide and nitrate. This might indicate that in the cleavage of casein with pepsin-hydrochloric acid, the phosphorus of the casein is removed in the form of a phosphorized body, leaving the thus non-phosphorized matter to break down into the caseoses. With this thought in mind, we propose to study later the nature and composition of the insoluble, semi-gelatinous body separated in the first stage of digestion. We also hope to extend our work by a study of Weyl's commercial "casein-peptone," preliminary examination of which has shown us the presence in large quantities of caseoses. In this way and by a somewhat different method of isolating the individual caseoses, we hope to verify our present work and at the same time obtain products comparatively free from ash, with which to establish beyond question the composition of the caseoses. We are also occupied in a study of pure casein-peptone, purified according to the method made use of by Kühne and Chittenden in the study of fibrin-peptone.

Influence of Some Organic and Inorganic Substances on Gas Metabolism. By R. H. Chittenden and G. W. Cummins, Ph.B.

While much time has been spent during the past few years in studying the influence of various substances on proteid metabolism, far less attention has been paid to the effects of these substances on the consumption of oxygen and the elimination of carbonic acid. Naturally in studying the influence of any substance on the nutrition of the body, we need to know not only its action on the excretion of nitrogen but also its influence on the production of carbonic acid. In this way only can we arrive at a true understanding of the influence of the substance on total metabolism, and obtain the necessary data from which to draw conclusions as to its influence on the consumption of either nitrogenous or non-nitrogenous matter. The difficulties, however, in the way of carrying on consecutive determinations of the relative amount of carbonic acid eliminated by the lungs are considerable, and in the absence of the necessary respiration apparatus, the difficulties are greatly increased. We have, however, endeavored to carry on some experiments in this direction, and although lacking the ordinary apparatus we have still been able with the means at our disposal to obtain some interesting results, a portion of which are simply confirmatory of previous work, while others are wholly new.

The apparatus employed in measuring the amount of carbonic acid eliminated is shown in the accompanying illustration (see Plate). The chamber in which the animal was placed during the experiment, was a bell jar of 32 litres capacity, with ground edge fitting closely upon a smooth glass plate. This when coated with grease made a perfectly tight joint, but in order to avoid any possibility of error, the jar and plate were placed in a shallow pan of galvanized iron, and water poured in to the depth of 2–3 inches, thus insuring a perfectly air-tight joint. In the top of the bell jar was an opening, closed with a doubly perforated rubber stopper, through which passed two tubes; one bringing air into the chamber, the other carrying it to the absorption apparatus. The inlet tube (to the left of the figure) was prolonged so as to admit the air nearly at the bottom of the jar, while the outlet tube came just through the stopper, thus insuring a perfect circulation of air. Air was drawn through the chamber by means of three aspirators,

two of which had a capacity of 15 litres and one of 7½ litres. The three aspirators working together would therefore draw through the chamber 37½ litres of air at every filling, and the flow was so regulated that 30 minutes were required to draw that amount of air through the apparatus. The flow of water from the aspirators was quite regular, since the inlet tubes went to the bottom and the air had to bubble up through the water, as the latter ran out, on the principle of Mariotte's bottle. The rate of flow was regulated by carefully changing the difference in height between the inlet tube (for air) of the aspirator and the outlet tube (for water). This of course, at the outset, was a tedious operation, but when once perfected and the apparatus permanently set up, the three aspirators ran exactly together, with a maximum variation of 15 seconds for the half-hour, which variation, however, was seldom observed. In addition, each aspirator was marked off into eight divisions, the last one of which was equal to only one-half of the others. In the two large aspirators these divisions indicated exactly the same volume, while in the small aspirator the divisions represented half the capacity of the former; but the flow of water in the latter was regulated to consume the same amount of time as in the former. Hence four minutes were required for the water to flow by each of the first seven divisions, and two minutes for the last, making a total of thirty minutes for the entire volume of water to flow from each aspirator.

The tube drawing the respired air from the chamber in which the animal was enclosed, was divided a short distance from the chamber, as seen in the figure, and two-fifths of the mixed air was drawn successively through three absorption tubes filled with a standard solution of barium hydroxide for absorption of the carbonic acid. The absorption tubes were about two-thirds of a metre long and the lower tube (*a*) contained 100 c. c. of a standard baryta solution, the middle tube (*b*) also 100 c. c. of the solution, and the upper tube (*c*) 50 c. c. The amount of carbonic acid absorbed was, at the end of the experiment, determined by titration with a standard solution of oxalic acid, using phenol-thalein as an indicator. Two titrations were made, one of the contents of tube *a* and one of the contents of the two tubes *b* and *c*. By using the three tubes, absorption of the carbonic acid was quite complete. In order to aid absorption, the air was broken into small bubbles by being forced through a small tube dipping beneath the barium hydroxide. Frequent blank experiments showed that all of the connections were perfectly tight, and further, all of the tubes being in the same position, that the flow of water

from each aspirator was perfectly uniform, and that the aspirators could be relied upon to draw the given volume of air through the apparatus in the time designated without any appreciable variation. In addition, the two-fifths drawn through the absorption tubes for determination of the carbonic acid was always exactly two-fifths of the aspirated air; since the aspirators, as already remarked, worked with perfect uniformity. Any tendency to variation, either in the time, or in the action of the individual aspirators, was noticed at the very outset of the experiment, as the water reached the level of the different marks on the aspirators, and could be at once checked or controlled by moving slightly the water outlet tube so as to either increase or diminish the difference in height between the latter and the inlet tube for air. Theoretically, variations in the temperature of the water in the aspirators might affect somewhat the volume of air analyzed, but a constant determination of the temperature of the water showed such slight variations that they did not seem to justify us in making any corrections for possible change in the amount of air aspirated. Naturally, all of the supports for the three absorption tubes were permanently placed, so that there could be no change of position; the tubes themselves placed in the same position in the holders; the volumes of baryta solution invariably the same, so as not to increase or decrease the pressure to be overcome; and lastly the aspirator tubes and stoppers fastened so as not to admit of any change. With these precautions, the results obtained, both as to the volume aspirated and the time consumed, were quite satisfactory.

As already mentioned, the total capacity of the three aspirators was 37½ litres or 5½ litres more than the capacity of the bell jar. This amount of air drawn through the chamber in 30 minutes, was more than enough to supply the largest rabbit experimented on, with the necessary amount of oxygen. But there must have been a slight accumulation of carbonic acid in the air of the chamber; this, however, was a constant factor throughout the experiments. Further, the results obtained, expressed in milligrams of CO_2, do not represent the total amount of carbonic acid eliminated by the rabbit during the thirty minutes of the experiment, but simply the amount of CO_2 contained in the 37½ litres of air aspirated during that time. Such a result, however, ought certainly to show just as plainly any influence on the elimination of carbonic acid, as a determination of absolute quantity and thus be equally valuable as an indication of influence or lack of influence on the gas metabolism of the body. Further, the results thus obtained ought to express equally as well, the comparative action of the various substances experimented with.

In every experiment, the time at which the animal was introduced into the bell jar was exactly noted and then two minutes were allowed before starting the aspirators, to make all of the connections properly. The rabbit was therefore under the bell jar, in each determination of carbonic acid, for exactly thirty-two minutes. The aspirators were started simultaneously and their progress carefully watched, in order to check any slight irregularity that might show itself.

The animals experimented with were wholly rabbits, and preliminary trials showed us plainly that it was very necessary to have them in a condition of hunger during the experiment, in order to avoid the irregularities incident to change in digestion. Further, we soon found that this was best accomplished by depriving the animal of food for three days, after which the experiment was commenced and allowed, as a rule, to extend through three consecutive days, the animal being deprived of food during the entire period. On the first of the three days, eight determinations of carbonic acid were made and the results obtained were used as a control, with which to compare the results obtained on the two following days, when the animal was being dosed with the substance experimented with. This, as a rule, we found to be the most satisfactory method of procedure, since small differences could not be relied upon as expressing anything of importance; for the varying restlessness of the confined animal, involving more or less muscular activity, would many times lead to variations in the amount of carbonic acid excreted, as may be noticed in the control experiments on those days when the animals were not dosed. Hence, the average of several consecutive results must necessarily express more correctly the average elimination of carbonic acid than any single result. Further, we deemed it better to allow the experiments to extend, as a rule, over several days and thus study the action of small, repeated doses of the various substances rather than to observe the effects of a single large dose, where violent action might naturally be expected.

The following table of results illustrates the way in which our experiments have been conducted, and at the same time shows the extent of variation, in the amount of carbonic acid, to be expected under normal circumstances from day to day. In this experiment, the rabbit had been deprived of food for three days, and the results show the amount of carbonic acid in the 37·5 litres of aspirated air for four distinct periods, during the fourth and fifth days. As already stated, the total amount of baryta solution employed in the three absorption tubes was 250 c. c., of which 100 c. c. were used in the first

or lower tube (a), while the remainder was used in the two other tubes b and c. Several solutions of oxalic acid were employed, the average strength of which was such that 1 c. c. equaled about 20 milligrams of carbonic acid.

Time.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. respirated air. mg.	Body temperature. ° C.
	tube a. c. c.	tubes b and c. c. c.						
March 18.								
9:48 to 10:18	10·4	25·0	35·4	45·1	9·7	180·2	450·5	38·8
11:51 to 12:21	10·6	25·2	35·8	45·1	9·3	172·7	432·0	38·1
2:59 to 3:29	10·5	25·3	35·8	45·1	9·3	172·7	432·0	38·2
5:03 to 5:33	10·4	24·9	35·3	45·1	9·8	182·0	455·1	38·3
March 19.								
8:59 to 9:29	10·2	25·1	35·3	45·1	9·8	182·0	455·1	38·3
10:53 to 11:23	10·9	25·3	36·2	45·1	8·9	165·3	413·4	37·7
2:53 to 3:23	10·2	24·2	34·4	45·1	10·7	198·7	496·8	38·7
4:51 to 5:21	10·6	24·9	35·5	45·1	9·6	178·3	445·9	38·9
Average,	10·5	25·0	35·5	45·1	9·6	179·0	447·6	38·4

Action of uranyl nitrate.

As stated in a preceding article,* the physiological action of uranium salts has been little studied. Experiments are now in progress to show the influence of uranium on proteid metabolism, and our present results show the influence of this substance on the excretion of carbonic acid. The rabbit first experimented with was deprived of food for three days, and on the fourth day the experiment was commenced, extending through three entire days, during which time the animal was without food. The accompanying tables show the results obtained. The body temperature was ascertained by inserting a self-registering thermometer into the rectum.

* Chittenden and Hutchinson, this volume.

A study of the first results shows plainly a decided action on the part of the uranium salt. The influence of the salt, however, manifests itself somewhat slowly, and it is not until the third day that its action becomes very pronounced, when the increased excretion of carbonic acid becomes very noticeable, accompanied with a slight rise in temperature. The first action of the uranium appears to cause a diminution in body temperature and in the amount of carbonic acid eliminated. The total amount of uranium salt given was quite large (1·175 grams in divided doses), and although no especial toxic symptoms showed themselves, the animal died on the day following the conclusion of the experiment.

A second series of experiments was tried, using smaller amounts of uranium nitrate and extending through four days, the results of which are also shown in the accompanying tables. The rabbit was deprived of food for four days prior to commencing the experiment. The amount of uranium nitrate given was considerably smaller than the quantity employed in the first series of experiments, and the animal did not suffer any permanent ill effects from its use. The following table shows the average daily result, expressed in milligrams of CO_2 contained in the 37·5 litres of aspirated air, together with the average body temperature.

May 3.	38·9° C.	574·3 milligrams	CO_2
" 4.	39·0	540·8 "	"
" 5.	39·9	581·2 "	"
" 6.	38·5	716·3 "	"

The uranium nitrate was introduced by hypodermic injection in the following quantities:

May 3.		0	
" 4.	5:18 p. m.	0·080 gram	of the salt.
" 5.	8:40 a. m.	0·090 "	"
" 5.	10:20 a. m.	0·100 "	"
" 5.	12:40 p. m.	0·150 "	"
" 5.	3:25 p. m.	0·150 "	"
" 5.	5:15 p. m.	0·200 "	"
" 6.		0	
		0·770	

In this second series of experiments it is to be noticed that the first two days are given up wholly to determining the normal excretion of carbonic acid, and the results show fully how close an agreement may be expected under normal circumstances. Taking the results

FIRST SERIES OF EXPERIMENTS WITH URANIUM.

Normal period, without uranium nitrate.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
April 19.								
A. M.								
9:47 to 10:17	6·5	24·8	31·3	46·3	15·0	278·9	697·3	38·7
10:45 to 11:15	7·8	25·8	33·6	46·3	12·7	235·3	588·3	38·7
11:46 to 12:16	7·9	25·8	33·7	46·3	12·6	233·4	583·6	38·6
P. M.								
2:04 to 2:34	8·4	26·3	34·7	46·3	11·6	214·9	537·3	38·4
2:57 to 3:27	8·4	26·1	34·5	46·3	11·8	218·6	546·6	38·7
3:58 to 4:28	8·2	26·1	34·3	46·3	12·0	221·4	553·5	38·6
4:52 to 5:22	8·5	26·2	34·7	46·3	11·6	214·9	537·3	38·7
Average,	7·9	25·9	33·8	46·3	12·5	231·0	577·7	38·9
April 20.			*With uranium nitrate.*					
A. M.								
9:04 to 9:34	7·3	25·9	33·2	46·3	13·1	242·7	606·8	38·4
9:57 to 10:27	8·3	26·8	35·1	46·3	11·2	207·5	518·8	38·4
10:59 to 11:29	9·8	26·6	36·4	46·3	9·9	183·4	458·6	38·2
11:53 to 12:23	8·7	26·3	35·0	46·3	11·3	209·3	523·5	38·1
P M.								
1:59 to 2:29	8·8	26·3	35·1	46·3	11·2	207·5	518·8	38·4
2:51 to 3:21	8·8	26·5	35·3	46·3	11·0	204·8	512·0	38·8
3:46 to 4:16	8·4	26·1	34·5	46·3	11·8	218·6	446·6	39·1
4:41 to 5:11	6·8	25·2	32·0	46·3	14·3	264·9	662·4	38·9
Average,	8·4	26·2	34·6	46·3	11·7	217·3	535·9	38·5

With uranium nitrate—continued.

Date. April 21.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
9:02 to 9:32	5·0	22·4	27·4	46·3	18·9	350·1	875·5	39·2
9:58 to 10:28	6·0	24·9	30·9	46·3	15·4	285·1	712·8	38·9
10:55 to 11:25	6·0	24·7	30·7	46·3	15·6	289·0	722·6	39·0
11·47 to 12:17	5·7	24·8	30·5	46·3	15·8	292·7	731·9	39·4
P. M.								
2:00 to 2:30	7·3	25·7	33·0	46·3	13·3	246·4	616·1	39·1
2:55 to 3:25	6·0	25·0	31·0	46·3	15·3	283·4	708·7	39·1
3:47 to 4:17	8·4	26·2	34·6	46·3	11·7	216·7	541·5	39·2
4:42 to 5:12	9·3	26·6	35·9	46·3	10·4	192·6	481·7	39·1
Average,	6·7	25·0	31·7	46·3	14·6	269·5	673·9	39·1

The following figures give the average daily result in body temperature and in the amount of carbonic acid contained in 37·5 litres of aspirated air:

April 19,	38·9° C.	577·7 milligrams CO_2
" 20,	38·5 "	535·9 " "
" 21,	39·1 "	673·9 " "

The uranium nitrate was introduced by hypodermic injection in the following quantities:

April 19,	5·40 p. m.	0·050 gram of the salt.
" 20,	8·55 a. m.	0·100 " "
" 20,	10·35 a. m.	0·100 " "
" 20,	12·40 p. m.	0·150 " "
" 20,	1·35 p. m.	0·150 " "
" 20,	5·30 p. m.	0·300 " "
" 21,	8·55 a. m.	0·200 " "
" 21,	2·45 p. m.	0·125 " "
		1·175

The animal died on the 22d.

SECOND SERIES OF EXPERIMENTS WITH URANIUM.

Normal period, without uranium.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air mg.	Body temperature. °C
	tube a. c. c.	tubes b and c. c. c.						
May 3.								
A. M.								
9:08 to 9:38	9·2	24·3	33·5	44·8	11·3	228·4	571·1	38·9
10:08 to 10:38	9·5	25·0	34·5	44·8	10·3	208·2	520·5	38·8
11:03 to 11:33	8·7	24·4	33·1	44·8	11·7	236·5	591·3	38·9
11:57 to 12:27	8·6	24·2	32·8	44·8	12·0	242·5	606·5	38·7
P. M.								
2:07 to 2:37	9·7	25·0	34·7	44·8	10·1	204·1	510·4	38·8
2:59 to 3:29	8·3	24·2	32·5	44·8	12·3	248·6	621·6	38·9
3:50 to 4:20	8·7	24·5	33·2	44·8	11·6	234·5	586·3	38·9
4:44 to 5:14	8·7	24·5	33·2	44·8	11·6	234·5	586·3	39·4
Average,	8·9	24·5	33·4	44·8	11·4	229·7	574·3	38·9
May 4.		*Normal period—continued.*						
A. M.								
8:50 to 9:20	9·2	24·7	33·9	44·8	10·9	220·3	550·9	38·9
9:49 to 10:19	9·5	24·4	33·9	44·8	10·9	220·3	550·9	38·9
10:44 to 11:14	8·9	24·6	33·5	44·8	11·3	228·4	571·1	38·9
11:37 to 12:07	9·5	24·8	34·3	44·8	10·5	212·2	530·6	38·9
P. M..								
1:56 to 2:26	9·1	25·0	34·1	44·8	10·7	216·3	540·8	39·1
2:48 to 3:18	9·6	25·2	34·8	44·8	10·0	202·1	505·6	39·2
3:41 to 4:11	9·4	25·0	34·4	44·8	10·4	210·2	525·6	39·1
4:32 to 5:02	9·3	24·8	34·1	44·8	10·7	216·3	540·8	39·0
Average,	9·3	24·8	34·1	44·8	10·7	216·3	540·8	39·0

With uranium nitrate.

Date. May 5.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
A. M.								
8:48 to 9:18	8·7	24·6	33·3	44·8	11·5	232·4	581·2	39·1
9:41 to 10:11	8·5	24·5	33·0	44·8	11·8	238·5	596·3	39·3
10:39 to 11:19	9·0	24·8	33·8	44·8	11·0	222·3	555·9	39·6
11:34 to 12:04	8·7	24·6	33·3	44·8	11·5	232·4	581·2	39·9
P. M.								
1:57 to 2:27	8·5	24·7	33·2	44·8	11·6	234·4	586·2	40·0
2:49 to 3:19	9·0	24·8	33·8	44·8	11·0	222·3	555·9	40·0
3:45 to 4:15	8·4	24·6	33·0	44·8	11·8	238·5	596·3	40·4
4:40 to 5:10	8·6	24·4	33·0	44·8	11·8	238·5	596 3	40·5
Average,	8·7	24·6	33·3	44·8	11·5	232·4	581·2	39·9
May 6.	*With uranium nitrate—continued.*							
A. M.								
8:52 to 9:22	7·0	22·1	29·1	44·8	15·7	317·3	793·4	40·0
9:52 to 10:22	5·6	23·3	28·9	44·8	15·9	321·4	803·5	39·6
10:48 to 11:18	6·9	24·0	30·9	44·8	13·9	280·9	702·5	38·2
11:43 to 12:13	6·9	24·0	30·9	44·8	13·9	280·9	702·5	37·9
P. M.								
2:11 to 2:41	7·5	24·6	32·1	44·8	12·7	256·7	641·8	37·4
3:02 to 3:32	7·5	24·4	31·9	44·8	12·9	261·7	654·4	38·1
Average,	6·9	23·7	30·6	44·8	14·2	286·5	716·3	38·5

EXPERIMENT WITH CUPRIC SULPHATE.

Normal period, without copper.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
May 10.	tube a. c. c.	tubes b and c. c. c.						
A. M.								
9:05 to 9:35	11·3	27·7	39·0	47·7	8·7	175·8	439·7	37·9
10:00 to 10:30	12·3	28·0	40·3	47·7	7·4	149·5	374·0	37·8
10:57 to 11:27	11·8	27·8	39·6	47·7	8·1	163·7	409·4	37·8
11:49 to 12:19	12·4	28·0	40·4	47·7	7·3	147·5	368·9	37·8
P. M.								
2:07 to 2:37	12·2	27·9	40·1	47·7	7·6	153·6	384·1	37·8
3:00 to 3:30	11·9	28·0	39·9	47·7	7·8	157·6	394·2	37·7
3:52 to 4:22	12·4	28·0	40·4	47·7	7·3	147·5	368·9	37·8
4:42 to 5:12	12·5	27·9	40·4	47·7	7·3	146·5	366·4	38·3
Average,	12·1	27·9	40·0	47·7	7·7	155·2	388·2	37·9
May 11.			*With cupric sulphate.*					
A. M.								
9:04 to 9:34	13·0	28·3	41·3	47·7	6·4	129·3	323·4	37·2
10:03 to 10:33	12·0	28·0	40·0	47·7	7·7	155·6	389·1	37·3
10:58 to 11:28	12·7	28·0	40·7	47·7	7·0	141·5	353·8	38·2
11:54 to 12:24	12·5	27·8	40·3	47·7	7·4	148·5	371·5	38·3
P. M.								
1:56 to 2:26	11·8	27·9	39·7	47·7	8·0	161·7	404·3	36·7
2:49 to 3:19	13·0	28·2	41·2	47·7	6·5	131·4	328·5	35·7
3:43 to 4:13	13·2	28·2	41·4	47·7	6·3	127·3	318·4	36·6
4:39 to 5:09	13·7	28·3	42·0	47·7	5·7	115·2	288·1	35·8
Average,	12·7	28·1	40·8	47·7	6·9	138·8	347·1	37·0

With cupric sulphate—Continued.

Date. May 12.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:11 to 9:41	13·3	28·3	41·6	47·7	6·1	123·3	308·3	35·2
10:06 to 10:36	13·6	28·3	41·9	47·7	5·8	117·2	293·1	35·3
11:00 to 11:30	14·7	28·3	43·0	47·7	4·7	95·0	237·5	35·7
11:52 to 12:22	13·6	28·4	42·0	47·7	5·7	115·2	288·1	35·8
P. M. 2:15 to 2:45	13·7	28·2	41·9	47·7	5·8	117·2	293·1	35·9
3:09 to 3:39	13·7	28·3	42·0	47·7	5·7	114·2	285·5	36·1
3:59 to 4:29	14·4	28·3	42·7	47·7	5·0	101·0	252.7	36·2
4:53 to 5:23	14·0	28·4	42·4	47·7	5·3	107·1	267·9	36·2
Average,	13·9	28·3	42·2	47·7	5·5	111·3	278·3	35·8

Following are the average daily results, expressed in milligrams of CO_2 contained in 37·5 litres of aspirated air, together with the average body temperature:

May 10.	37·9° C.	388·2 milligrams CO_2
" 11.	37·0	347·1 " "
" 12.	35·8	278·3 " "

The cupric sulphate was introduced by hypodermic injection, in the following amounts:

May 10.	5·34 p. m.	0·025 gram $CuSO_4$
" 11.	8:57 a. m.	0·005 " "
" 11.	9:55 a. m.	0·025 " "
" 11.	12:29 p. m.	0·050 " "
" 12.	9:08 a. m.	0·025 " "
		0·130

Rabbit died on the 13th.

of these two days for comparison, it is seen that the action of the uranium is somewhat slow, but that it produces on the first day (May 5) a noticeable rise in temperature, without any appreciable change in the elimination of carbonic acid. The full effect of the uranium, however, shows itself on the day following the last dose of the salt, and we then see the same noticeable increase in the elimination of carbonic acid noticed in the first series of experiments. We have to conclude, then, that uranium nitrate, when taken in sufficient quantity, tends to raise materially the body temperature and to increase very noticeably the excretion of carbonic acid.

Action of cupric sulphate.

Falck, as quoted by Dr. H. C. Wood,* has found that cupric sulphate acts upon dogs, pigeons, rabbits and similar animals as an irritant, neurotic poison; producing great depression of temperature, with progressive general paresis ending in death, apparently from failure of respiration. Our experiments on rabbits show a marked influence of the salt in depressing body temperature and a still greater influence in diminishing the production of carbonic acid. The results of one experiment are shown in the preceding table. Although but 130 milligrams of the copper salt were used altogether, the animal finally died on the day following the conclusion of the experiment.

Action of arsenious oxide.

C. Schmidt and Stürzwage† have shown by experiments on cats, that arsenious acid tends to produce a noticeable diminution in the excretion of both nitrogen and carbonic acid. Voit, however, has pointed out that in these experiments, the diminished excretion depends simply on the loss of a large portion of the food by vomiting, and Bolck‡ has shown that small doses of arsenious oxide given to hungry dogs, is wholly without influence on the decomposition of proteid matter. With large, toxic doses of arsenic, Gähtgens§ and Kossel‖ have shown that a very noticeable increase in the elimination of nitrogen takes place. These facts constitute about the sum

* Therapeutics, Materia Medica and Toxicology, p. 46.
† Moleschott's Untersuchungen, vi, p. 283.
‡ Zeitschrift für Biologie, vii, p. 430.
§ Centralblatt f. Med. Wissen., 1875, p. 529.
‖ Archiv. f. exper. Path. u. Pharm, v, p. 128.

total of our knowledge regarding the action of arsenic on tissue changes.

Our experiments were made with rabbits in a condition of hunger, deprived of food for three days prior to the experiment, and the results appear to show that arsenious acid, in the case of rabbits, has a tendency to diminish the excretion of carbonic acid, presumably through its action on the metabolic activity of the tissue cells. The amount of arsenic given was quite small and the animal seemed wholly unaffected by the poison.

Action of potassium antimony tartrate.

Voit* states that antimony in large doses affects proteid metabolism in the same manner as arsenic, and since Saikowsky has shown that both arsenic and antimony tend to produce a fatty degeneration of the various organs, in which presumably the fat is formed from the decomposition of proteid matter, the non-nitrogenous moiety of the albumin molecule being stored up as fat instead of being burned to carbonic acid, it seems natural to expect that these two substances when taken in large quantity at least, should like phosphorus diminish both the consumption of oxygen and the elimination of carbonic acid.

With rabbits our results with antimony certainly lead to this conclusion. Even small doses of tartar emetic quickly lead to a diminished excretion of carbonic acid and also to a noticeable fall in temperature. In the first series of experiments, the results of which are shown in the accompanying tables, the excretion of carbonic acid fell from 363·6 milligrams per 37·5 litres of aspirated air to 203·8 milligrams and with a fall in temperature of from 39° C. to 34·6° C. The total amount of tartar emetic given was 82 milligrams.

In the second series of experiments, where as before, the rabbit had been deprived of food for three days prior to the experiment, still smaller quantities of antimony were given with even more pronounced results, both in the diminution of carbonic acid and in the depression of temperature. Thus while in the normal period the excretion of carbonic acid amounted to 396 milligrams per 37·5 litres of aspirated air and with a normal temperature of 38·6° C., tartar emetic (0·055 gram) given in divided doses reduced the carbonic acid to 106·5 milligrams per 37·5 litres of aspirated air and the temperature to 27·0° C. Ackermann† has already called attention to the great decrease in animal heat produced by antimony, notably in the case of rabbits.

* Hermann's Handbuch der Physiologie, Band vi, p. 184.

† See H. C. Wood, Therapeutics, etc., p. 158.

EXPERIMENT WITH ARSENIOUS OXIDE.

Normal period, without arsenic.

Date. June 7.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
10:04 to 10:34	7·8	24·5	32·3	44·3	12·0	242·5	606·5	38·7
11:08 to 11:38	7·5	25·2	32·7	45·5	12·8	258·7	646·9	38·9
12:06 to 12:36	9·2	26·2	35·4	45·5	10·1	204·1	510·4	38·9
P. M.								
1:57 to 2:27	8·3	25·6	33·9	45·5	11·6	234·5	586·3	38·9
2:52 to 3:22	8·6	25·7	34·3	45·5	11·2	226·4	566·0	39·0
3:50 to 4:20	6·9	25·5	32·4	45·5	13.1	264·8	662·0	39·3
4:44 to 5:14	9·1	24·9	34·0	45·5	10·5	211·2	528·1	38·9
Average,	8·2	25·4	33·6	45·3	11·6	234·6	586·6	38·9
June 8.			*With arsenious oxide.*					
A. M.								
9:00 to 9:30	9·0	25·8	34·8	45·5	10·7	215·2	538·2	38·8
9:52 to 10:22	10·3	26·4	36·7	45·5	8·8	177·8	444·7	38·7
10:45 to 11:15	10·1	26·3	36·4	45·5	9·1	183·9	459·9	38·8
11:37 to 12:07	9·8	26·4	36·2	45·5	9·3	188·0	470·0	38·8
P. M.								
2:14 to 2:44	9·1	26·0	35·1	45·5	10·4	218·3	545·8	39·0
3:08 to 3:38	10·4	26·5	36·9	45·5	8·6	172·8	432·1	39·0
3:58 to 4:28	10·0	26·4	36·4	45·5	9·1	182·9	457·4	39·2
4:48 to 5:18	9·4	26·3	35·7	45·5	9·8	198·1	495·3	39·2
Average,	9·8	26·3	36·0	45·5	9·5	192·1	480·4	38·9

With arsenious oxide—continued.

Date. June 9.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
	tube a. c. c.	tubes b and c. c. c.						
A. M.								
9:00 to 9:30	9·4	26·1	35·5	45·5	10·0	202·1	505·4	39·0
9:52 to 10:22	10·4	26·5	36·9	45·5	8·6	172·8	432·1	38·9
10:41 to 11:11	9·1	26·1	35·2	45·5	10·3	208·2	520·5	39·1
11:36 to 12:06	9·6	26·3	35·9	45·5	9·6	194·0	485·2	39·0
P. M.								
2:15 to 2:45	9·6	26·5	36·1	45·5	9·4	190·0	475·1	39·2
3:08 to 3:38	9·6	26·5	36·1	45·5	9·4	190·0	475·1	38·9
3:57 to 4:27	10·3	26·5	36·8	45·5	8·7	175·8	439·7	39·2
4:47 to 5:17	10·0	26·5	36·5	45·5	9·0	181·9	454·8	39·2
Average,	9·7	26·4	36·1	45·5	9·4	189·3	473·5	39·1

Average daily excretion of carbonic acid expressed in milligrams of CO_2 per 37·5 litres of aspirated air, and average temperature is as follows :

June 7	38·9° C.	586·6	milligrams CO_2
" 8	38·9	480·4	" "
" 9	39·1	473·5	" "

The arsenious oxide was introduced by way of the mouth in small gelatin capsules, in the following doses :

June 7	5:25 p. m.	0·005	gram As_2O_3
" 8	8:47 a. m.	0·005	" "
" 8	12:12 p. m.	0·005	" "
" 8	5:25 p. m.	0·005	" "
" 9	8:50 a. m.	0·005	" "
" 9	12:12 p. m.	0·010	" "
		0·035	

FIRST SERIES OF EXPERIMENTS WITH ANTIMONY.

Normal period, without tartar emetic.

Date. March 31.	Oxalic acid to neutralize baryta solution. tube a. c. c.	tubes b and c. c. c.	Total oxalic acid used. c c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c. oxalic acid.	CO_2 in a. b and c. mg.	CO_2 in 37·5 L. aspirated air.	Body temperature. °C.
A. M.								
9:03 to 9:33	13·9	30·9	44·8	52·6	7·8	144·5	361·3	38·6
10:01 to 10:31	13·8	31·0	44·8	52·6	7·8	144.5	361·3	38·8
10:56 to 11:26	15·0	31·0	46·0	52·6	6·6	122·2	305·7	38·9
11:52 to 12:22	13·7	30·8	44·5	52·6	8·1	150·0	375·2	39·2
P. M.								
1:55 to 2:25	12·5	30·0	42·5	52·6	10·1	187·0	467·7	39·1
2:47 to 3:17	15·0	31·1	46·1	52·6	6·5	120·4	301·1	39·2
3:41 to 4:11	14·4	30·4	44·8	52·6	7·8	144·5	361·3	39·2
4:36 to 5:06	13·7	30·8	44·5	52·6	8·1	150·0	375·2	39·3
Average,	14·0	30·7	44·7	52·6	7.85	145·4	363·6	39·0
April 1.			*With tartar emetic.*					
A. M.								
8:57 to 9:27	14·1	30·8	44·9	52·6	7·7	141·7	354·4	38·1
9:54 to 10:24	14·5	31·0	45·5	52·6	7·1	131·5	328·9	36·9
10:50 to 11:20	15·4	31·0	46·4	52·6	6·2	114·8	287·2	36·4
11:44 to 12:24	15·4	31·0	46·4	52·6	6·2	114·8	287·2	35·7
P. M.								
2:01 to 2:31	17·0	31·2	48·2	52·6	4·4	81·5	203·8	34·6
Average,	15·3	31·0	46·3	52·6	6·3	116·9	292·3	36·3

The antimony was given in the form of tartar emetic and was introduced by hypodermic injection as follows:

March 31.	5:20 p. m.	0·012 gram	tartar emetic.
April 1.	8:45 a. m.	0·035 "	"
" 1.	12:43 p. m.	0·035 "	"
		0·082	

Rabbit died at 3:30 p. m., April 1.

SECOND SERIES OF EXPERIMENTS WITH ANTIMONY.

Normal period, without tartar emetic.

Date. April 5.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
A. M.								
9:14 to 9:44	14·4	30·9	45·3	52·6	7·3	135·2	338·1	38·4
10:09 to 10:39	13·0	30·3	43·3	52·6	9·3	172·3	430·8	38·5
11:04 to 11:34	13·1	30·5	43·6	52·6	9·0	166·7	416·9	38·4
11:57 to 12:27	14·5	30·8	45·3	52·6	7·3	135·2	338·1	38·6
P. M.								
2:05 to 2:35	13·8	30·7	44·5	52·6	8·1	150·0	375·2	38·9
2:58 to 3:28	13·1	30·5	43·6	52·6	9·0	166·7	416·9	38·8
3:52 to 4:22	12·7	30·5	43·2	52·6	9·4	174·1	435·4	38·9
4:45 to 5:15	12·9	30·7	43 6	52·6	9·0	166·7	416·9	38·7
Average,	13·4	30·6	44·0	52·6	8·5	158·4	396·0	38·6
April 6			*With tartar emetic.*					
A. M.								
8:59 to 9:29	14·5	30·8	45·3	52·6	7·3	135·2	338·1	37·4
9:57 to 10:27	15·2	31·1	46·3	52·6	6·3	116·7	291·8	36·7
10:52 to 11:22	14·5	30·6	45·1	52·6	7·5	138·9	347·4	36·6
11:46 to 12:16	14·5	31·0	45·5	52·6	7·1	131·5	328·9	37·3
P. M.								
1:58 to 2:28	15·3	30·9	46·2	52·6	6·35	117·6	294·1	36·2
2:50 to 3:20	14·5	30·9	45·4	52·6	7·2	133·4	333·5	36·2
3:42 to 4:12	15·4	31·2	46·6	52·6	5·95	110·2	275·6	35·4
4:38 to 5:08	15·7	31·2	46·9	52·6	5·7	115·6	289·0	35·7
Average,	14·9	31·0	45·9	52·6	6·68	124·9	312·2	36·7

With tartar emetic—continued.

Date. April 7.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:00 to 9:30	17·6	31·2	48·8	52·6	3·8	70·4	176·0	30·0
9:59 to 10:29	18·5	31·3	49·8	52·6	2·8	51·8	129·7	28·0
10:58 to 11:23	18·9	31·4	50·3	52·6	2·3	42·6	106·5	27·0
Average,	18·3	31·3	49·6	52·6	2·97	54·9	137·4	28·3

Average daily excretion of carbonic acid expressed in milligrams of CO_2 per 37·5 litres of aspirated air, together with average temperature is as follows:

April 5.	38·6° C.	396·0 milligrams	CO_2
6.	36·7	312·0 "	"
7.	28·3	137·4 "	"

The following amounts of antimony were injected:

April 5.	5:30 p. m.	0·015 gram	tartar emetic.
6.	8:45 a. m.	0·015 "	"
6.	12:39 p. m.	0·015 "	"
6.	5:24 p. m.	0·010 "	"
		0·055	

Rabbit died at 12 m., April 7.

Action of morphine sulphate.

Boeck and Bauer* have already made a careful study of the action of morphine on the elimination of carbonic acid and the absorption of oxygen. By experiments on a cat and on a dog they found that the action of morphine on metabolism was mainly an indirect one, affecting especially the consumption of non-nitrogenous matter. Further, that its action hinged mainly on its power of affecting muscular activity; thus in the case of a cat the first action of morphine was to increase the elimination of carbonic acid and the consumption of oxygen, due to the increased muscular activity induced by the poison, while in the case of a dog, where narcosis was half induced, there was a diminution in the amount of carbonic acid eliminated amounting in one case to 27 per cent. This diminished excretion was due almost wholly to the quieting action of the morphine and was followed by an after period in which there was increased production of carbonic acid, due to the increased activity of the muscle tissue.

In these experiments the dose of morphine was 0·05 gram, in the form of chloride, and was introduced by subcutaneous injection. The injection of the poison was followed soon after by convulsions, etc., indicating vigorous toxic action. In our first series of experiments we endeavored to have the toxic action less pronounced, and for this reason the morphine was introduced by way of the mouth in repeated doses, the experiment extending through three days and into the fourth. The rabbit was deprived of food through the entire period and had also been kept without food for three days prior to the experiment. The data are to be found in the accompanying tables.

The results do not show any very marked action, either on the excretion of carbonic acid or on the body temperature. At no time was there any noticeable indication of increased muscular activity, the rabbit remaining fairly quiet in the chamber and showing no symptoms of tetanic convulsions. On the other hand there was no very profound narcotism. A study of the individual results, however, shows that directly after each dose of morphine, the excretion of carbonic acid fell quite noticeably for one or two periods. Such action as was produced, therefore, in this experiment, is to be considered simply as incidental to the semi-somnolent condition of the animal.

In a second shorter series of experiments with a rabbit, one single

* Zeitschrift für Biologie, Band x, p. 339.

FIRST SERIES OF EXPERIMENTS WITH MORPHINE.

Normal period, without morphine.

Date. March 24.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
8:54 to 9:24	11.0	25·5	36·5	45·1	8·6	159·8	399·5	37·9
9:53 to 10:23	11·9	25·8	37·7	45·1	7·4	137·5	344·0	38·0
10:50 to 11:20	11·6	25·7	37·3	45·1	7·8	144·9	362·5	37·9
11:46 to 12:16	....	....	36·8	45·1	8·3	154·2	385·7	38·0
P. M.								
2:14 to 2:44	10·6	26·1	36·7	45·1	8·4	156·1	390·3	38·2
3:08 to 3:38	10·2	26·1	36·3	45·1	8·8	163·5	408·8	38·3
4:02 to 4:32	10·3	26·1	36·4	45·1	8·7	161·6	404·2	38·2
4:55 to 5:25	11·4	26·1	37·5	45·1	7·6	141·2	353·2	37·8
Average,	11·0	25·9	36·9	45·1	8·2	152·4	381·0	38·0
March 25.			*With morphine sulphate.*					
A. M.								
8:51 to 9:21	11·7	26·5	38·2	45·1	6·9	128·3	320·8	38·2
9:53 to 10:23	11·8	26·5	38·3	45·1	6·8	126·4	316·2	37·8
10:47 to 11:17	10·9	26·2	37·1	45·1	8·0	148·7	371·8	38·0
11:44 to 12:14	11·1	26·4	37·5	45·1	7·6	141·2	353·2	38·0
P. M.								
2:01 to 2:31	11·1	26·2	37·3	45·1	7.8	144·0	360·1	38·7
3:08 to 3:38	11·7	26·2	37·9	45·1	7·2	133·8	334·7	38·7
4:04 to 4:34	11·0	25·7	36·7	45·1	8·4	156·1	390·3	38·6
4:57 to 5:27	10·0	25·2	35·2	45·1	9·9	184·9	462·3	38·8
Average,	11·1	26·1	37·2	45·1	7·9	145·4	363·7	38.4

With morphine sulphate—continued.

Date. March 26.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
A. M.								
9:24 to 9:54	17·6	35·8	53·4	60·5	7·1	131·5	328·9	38·8
10:19 to 10:49	16·4	35·6	52·0	60·5	8·5	157·4	393·7	38·8
11:14 to 11:44	16·5	35·7	52·2	60·5	8·3	153·7	384·5	38·6
12:09 to 12:39	16·5	35·7	53·2	60·5	8·3	153·7	384·5	38·9
P. M.								
1:58 to 2:28	17·4	35·8	53·2	60·5	7·3	135·2	338·1	38·9
2:55 to 3:25	17·0	35·7	52·7	60·5	7·8	144·5	361·3	38·7
3:49 to 4:19	16·1	35·5	51·6	60·5	8·9	164·9	412·3	38·9
4:48 to 5:18	16·0	35·3	51·3	60·5	9·2	170·4	423·7	38·8
Average,	16·7	35·6	52·3	60·5	8·2	151·3	378·4	38·8
March 27.								
A. M.								
8:33 to 9:03	16·2	35·6	51·8	60·5	8·7	161·1	403·0	38·9
9:30 to 10:00	15·7	35·5	51·2	60·5	9·3	172·3	430·8	38·9

Following is the average daily excretion of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature:

March 24.	38·0° C.	381·0 milligrams	CO_2
" 25.	38·4	363·7 "	"
" 26.	38·8	378·4 "	"
" 27.	38·9	416·9 "	"

The morphine was introduced into the stomach in solution in the following amounts:

March 24.	6:00 p. m.	0·075	gram	morphine sulphate.
" 25.	8:35 a. m.	0·075	"	"
" 25.	12:35 p. m.	0·075	"	"
" 25.	5:50 p. m.	0·100	"	"
" 26.	9:15 a. m.	0·100	"	"
" 26.	12:50 p. m.	0·100	"	"
		0·525		

large dose of morphine sulphate was given, and given by hypodermic injection. In this case, as before, there was no increased muscular activity, but there was a sudden and rapid fall both in temperature and in the amount of carbonic acid excreted, the latter of which was still quite pronounced on the following day, although the temperature has gone back to normal. This diminution in carbonic acid was accompanied by a profound narcotism, the animal lying almost motionless and with a respiration ranging from 8 to 20 per minute. The results, which are to a certain extent corroborative of Boeck and Bauer's, are shown in the accompanying table:

SECOND SERIES OF EXPERIMENTS WITH MORPHINE.

Normal period without morphine sulphate.

Time.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivaleut to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
March 12.								
A. M.								
9:12 to 9:42	15.4	34·1	49·5	59·25	9·75	180·6	465·0	39·1
10:00 A. M., injected subcutaneously 0·100 gram morphine sulphate.								
A. M.								
10:23 to 10:53	16·9	34·5	51·4	59·25	7·85	145·4	363·5	36·6
11:29 to 11:59	17·3	34·5	51·8	59·25	7·45	138·0	342·5	34·4
12:32 to 1:02	16·3	34·1	50·4	59·25	8·85	164·0	410·0	33·3
P. M.								
2:48 to 3:18	17·0	34·2	51·2	59·25	8·05	149·2	373·0	34·4
3:49 to 4:19	17·8	34·6	52·4	59·25	6·85	126·9	317·3	35·0
4:47 to 5:17	17·1	34·3	51·4	59·25	7·85	145·4	363·5	36·3
March 13.								
10:08 to 10:38	16·9	34·3	51·2	59·25	8·05	149·2	373·0	39·2

Action of quinine sulphate.

The importance of quinine as a therapeutic agent and particularly its value as a febrifuge has led to a thorough study of its physiolog-

ical action. In this connection, the action of quinine on proteid metabolism has been very thoroughly investigated, but as to its exact influence on the decomposition of non-nitrogenous matter, as shown by its effects on the elimination of carbonic acid, there is less unanimity of opinion. This is naturally a point of considerable importance for if, as is generally supposed, the alkaloid has the power of diminishing body temperature, it would presumably be due to its influence on the combustion of non-nitrogenous matter in the body. Ranke, Kerner, von Boeck and others have plainly shown the power of quinine to diminish proteid metabolism, but Strassburg, by an elaborate series of experiments* found that the alkaloid had no very decided effect upon the elimination of carbonic acid, either in healthy or fevered rabbits.

Boeck and Bauer,† however, from experiments on cats, claim that quinine in the first stage of its action diminishes somewhat the production of carbonic acid, owing to its inhibitory action on the tissue cells; but when large doses of quinine are given, so that convulsions appear, then there is an increased production of carbonic acid, owing to the greater decomposition of non-nitrogenous matter incident to increased muscular activity. With small doses of the alkaloid, it is to be presumed that the slight diminution in carbonic acid noticed by Boeck and Bauer comes simply from diminished proteid metabolism.

In our experiments, rabbits only were used and these in a condition of hunger, having been deprived of food for three days prior to the experiment. In the first series of experiments, the total amount of quinine given was quite large, so that at last the animal finally died from its effects. No decided action on the production of carbonic acid was noticed until just before the animal's death, when both the body temperature and the amount of carbonic acid fell quite noticeably. On the second day of the experiment, when the quinine was first being given, the body temperature, as taken per rectum, fell quite gradually until it finally reached a point 1·5 °C. below the average of the normal period. The results of the experiment are to be seen in the accompanying tables. The quinine given was in the form of hard, gelatin-coated pills and possibly was not as rapidly absorbed as might otherwise have been. At no time was the rabbit in convulsions.

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* Quoted from Dr. H. C. Wood, Therapeutics, p. 75.

† Zeitschrift für Biologie, Band x, p. 350.

FIRST SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Date. April 12.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
9:11 to 9:41	9·5	24·2	33·7	41·5	7·8	144·5	361·5	38·7
10:05 to 10:35	10·8	24·4	35·2	41·5	6·3	116·7	291·8	38·7
10:58 to 11:28	10·8	24·2	35·0	41·5	6·5	120·4	301·1	38·7
11:54 to 12:24	10·7	24·2	34·9	41·5	6·6	122·2	305·7	38·3
P. M.								
1:58 to 2:28	10·0	24·0	34·0	41·5	7·5	138·9	347·4	38·6
2:51 to 3:21	10·0	24·2	34·2	41·5	7·3	135·2	338·1	38·4
3:49 to 4:19	10·5	24·3	34·8	41·5	6·7	124·1	310·4	38·7
4:41 to 5:11	11·0	24·3	35·3	41·5	6·2	114·8	287·2	38·8
Average,	10·4	24·2	34·6	41·5	6·8	127·1	317·8	38·6
April 13.		*With quinine sulphate.*						
A. M.								
9:14 to 9:44	9·3	23·9	33·2	41·5	8·3	153·7	384·5	38·6
10:10 to 10:40	11·3	23·9	35·2	41·5	6·3	116·7	291·8	38·6
11:05 to 11:35	11·0	24·4	35·4	41·5	6·1	113·0	282·6	38·2
11:58 to 12:28	10·3	24·2	34·5	41·5	7·0	129·7	314·3	38·2
P. M.								
1:55 to 2:25	9·4	24·0	33·4	41·5	8·1	150·0	375·2	38·1
2:46 to 3:16	10·1	24·2	34·3	41·5	7·2	133·4	333·3	37·6
3:40 to 4:10	9·3	23·8	33·1	41·5	8·4	168·7	340·5	37·1
4:32 to 5:02	10·3	24·1	34·4	41·5	7·1	143·5	282·6	38·0
Average,	10·1	24·1	34·2	41·5	7·3	138·6	325·6	38·1

With quinine sulphate—continued.

Date. April 14.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
8:54 to 9:24	10·0	24·3	34·3	41·5	7·2	133·4	333·5	38·6
9:50 to 10:20	10·2	24·1	34·3	41·5	7·2	133·4	333·5	38·2
10:43 to 11:13	9·8	24·0	33·8	41·5	7·7	142·6	356·7	38·3
11:38 to 12:08	10·9	24·3	35·2	41·5	6·3	116·7	291·8	37·6
P. M.								
1:57 to 2:27	11·3	24·5	35·8	41·5	5·7	105·6	264·0	34·7
Average,	10·4	24·2	34·6	41.5	6·9	126·3	315·9	37·5

The following amounts of quinine were given by way of the mouth:

April 12	5:20 p. m.	0·130	gram	quinine	sulphate.
" 13	9:10 a. m.	0·260	"	"	"
" 13	1:00 p. m.	0·390	"	"	"
" 13	5:15 p. m.	0·520	"	"	"
" 14	8:45 a. m.	0·520	"	"	"
" 14	12:00 m.	0·780	"	"	"
		2·600			

Rabbit died at 3 p. m. April 14.

Following are the average daily results in temperature and in the amount of carbonic acid excreted per 37·5 litres of aspirated air.

April 12	38·6° C.	317·8	milligrams	CO_2.
" 13	38·1 "	325·6	"	"
" 14	37·5 "	315·9	"	"

SECOND SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Date. May 17.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
8:40 to 9:10	5·3	22·2	27·5	41·8	14·3	289·0	722·7	38·8
9:33 to 10:03	6·1	22·8	28·9	41·8	12·9	260·7	651·9	38·8
10:29 to 10:59	6·2	22·6	28·8	41·8	13·0	262·7	657·0	38·8
11:24 to 11:54	4·9	21·9	26·8	41·8	15·0	303·2	758·1	39·1
P. M.								
1:55 to 2:25	5·0	22·1	27·1	41·8	14·7	297·1	742·9	39·0
2:48 to 3:18	4·5	22·0	26·5	41·8	15·3	309·2	773·2	38·9
3:43 to 4:13	6·7	23·1	29·8	41·8	12·0	242·5	606·5	38·8
4:35 to 5:05	6·7	23·0	29·7	41·8	12·1	244·5	611·5	38·9
Average,	5·7	22·4	28·1	41·8	13·7	276·8	690·5	38·6
May 18.		*With quinine sulphate.*						
A. M.								
9:05 to 9:35	6·2	22·5	28·7	41·8	13·1	264·8	662·0	38·6
10:00 to 11:30	7·5	23·0	30·5	41·8	11·3	228·4	571·1	38·4
10:51 to 11:21	5·6	22·1	27·7	41·8	14·1	285·0	712·6	38·3
11:46 to 12:16	6·0	22·5	28·5	41·8	13·3	268·8	672·2	38·8
P. M.								
2:14 to 2:44	6·5	22·8	29·3	41·8	12·5	252·6	631·7	38·9
3:07 to 3:37	6·5	22·9	29·4	41·8	12·4	249·6	624·1	39·0
4:01 to 4:31	7·0	23·1	30·1	41·8	11·7	236·5	591·3	39·0
4:58 to 5:28	6·0	22·1	28·1	41·8	13·7	276·9	692·4	38·4
Average,	6·4	22·6	29·0	41·8	12·8	257·8	644·7	38·7

With quinine sulphate—continued.

Date. May 19.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
9:02 to 9:32	7·3	23·0	30·3	41·8	11·5	232·4	581·2	38·9
9:57 to 10:27	7·6	23·2	30·8	41·8	11·0	222·3	555·9	38·4
10:50 to 11:20	7·2	23·1	30·3	41·8	11·5	232·4	581·2	38·7
11:43 to 12:13	6·9	23·0	29·9	41·8	11·9	240·5	601·4	38·6
P. M.								
2:03 to 2:33	7·5	23·2	30·7	41·8	11·1	223·3	558·4	38·9
2:54 to 3:24	8·2	23·6	31·8	41·8	10·0	202·1	505·4	38·5
3:47 to 4:17	7·4	23·3	30·7	41·8	11·1	224·3	561·0	38·8
4:40 to 5:10	7·1	23·1	30·2	41·8	11·6	234·5	586·3	38·8
Average,	7·4	23·2	30·6	41·8	11·2	226·4	566·4	38·7

The quinine was given by way of the mouth in gelatin capsules, in the following quantities:

May 17	5:30 p. m.	0·250 gram quinine sulphate.
" 18	8:50 a. m.	0·250 " " "
" 18	12:30 p. m.	0·250 " " "
" 18	5:35 p. m.	0·250 " " "
" 19	8:50 a. m.	0·325 " " "
" 19	1:55 p. m.	0·250 " " "
		1·575

Following are the average daily excretions of carbonic acid expressed in milligrams of CO_2 per 37·5 litres of aspirated air, together with the average body temperature:

May 17	38·6° C.	690·5 milligrams CO_2
" 18	38·7 "	644·7 " "
" 19	38·7 "	566·4 " "

THIRD SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Time. March 8.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:50 to 10:20	15·5	33·9	49·4	59·25	9·85	182·5	456·3	39·2
10:58 A. M. injected subcutaneously 0·083 gram quinine sulphate.								
11:15 to 11:45	16·9	34·5	51·4	59·25	7·85	145·4	363·5	38·4
12:25 to 12:55	15·3	33·9	49·2	59·25	10·05	186·2	465·5	39·9
P. M. 3:00 to 3:30	15·6	34·0	49·6	59·25	9·65	178·8	447·0	39·8
4:06 to 4:36	15·8	33·7	49·5	59·25	9·75	180·7	451·8	39·9
Average,	15·9	34·0	49·9	59·25	9·35	172·8	432·0	39·5

FOURTH SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Time. March 10.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:11 to 9:41	15·0	33·8	48·8	59·25	10·45	193·6	484·0	38·8
10:15 A. M. injected subcutaneously 0·15 gram quinine sulphate.								
10:33 to 11:03	16·5	34·6	51·1	59·25	8·15	151·0	377·5	38·4
11:39 to 12:09	15·4	33·9	49·3	59·25	9·95	184·4	461·0	38·9
12:38 to 1:08	15·3	34·1	49·4	59·25	9·85	182·5	456·3	38·4
P. M. 2:54 to 3:24	16·0	34·3	50·3	59·25	8·95	165·8	414·5	39·1
4:07 to 4:37	15·9	34·1	50·0	59·25	9·25	171·4	428·5	38·8
Average,	15·8	34·2	50·0	59·25	9·25	171·0	427·6	38·7

EXPERIMENT WITH CINCHONIDINE.

Normal period, without cinchonidine sulphate.

Date. June 24.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. ° C.
A. M.								
9:00 to 9:30	10·6	28·8	39·4	50·2	10·8	218·3	545·8	36·4
9:56 to 10·26	11·2	29·1	40·3	50·2	9·9	200·1	500·3	37·4
10:47 to 11:17	12·0	29·0	41·0	50·2	9·2	185·9	465·0	38·1
11:38 to 12:08	10·6	28·7	39·3	50·2	10·9	220·3	550·9	36·3
P. M.								
2:03 to 2:33	10·3	28·5	38·8	50·2	11·4	230·4	576·1	36·9
2:51 to 3:21	9·4	28·2	37·6	50·2	12·6	254·9	636·8	37·1
3:42 to 4:12	9·7	28·5	38·2	50·2	12·0	242·5	606·5	37·0
4:30 to 5:00	9·7	28·5	38·2	50·2	12·0	242·5	606·5	36·4
Average,	10·5	28·6	39·1	50·2	11·1	224·3	561·0	37·0
June 25.		*With cinchonidine sulphate.*						
A. M.								
9:03 to 9:33	10·8	28·6	39·4	50·2	10·8	218·3	545·8	38·6
9:58 to 10:28	11·4	28·9	40·3	50·2	9·9	200·1	500·3	38·6
10:53 to 11:23	11·1	28·7	39·8	50·2	10·4	210·2	525·6	38·6
11:48 to 12:18	11·6	28·7	40·3	50·2	9·9	200·1	500·3	38·7
P. M.								
2:04 to 2:34	11·1	28·8	39·9	50·2	10·3	208·2	520·5	38·9
2:59 to 3:29	11·5	29·0	40·5	50·2	9·7	196·0	490·2	39·0
3:56 to 4:26	11·6	29·0	40·6	50·2	9·6	194·0	485·2	39·0
4:50 to 5:20	12·1	29·1	41·2	50·2	9·0	181·9	454·8	39·1
Average,	11·4	28·8	40·2	50·2	10·0	201·1	502·8	38·8

Following are the average daily amounts of carbonic acid excreted, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature.

June 24, 37·0° C., 561·0 milligrams CO_2.
" 25, 38·8 502·8 " "

Following are the doses of cinchonidine :

June 24, 5:12 P. M., 1·000 gram cinchonidine sulphate.
" 25, 10:35 A. M., 0·250 " " "
" 25, 12:35 P. M., 0·325 " " "
1·575

Rabbit died at 5:40 P. M., June 25, in convulsions.

In the second series of experiments, smaller doses of quinine were employed, so that no special toxic action was observed. In this case there seemed to be a gradual falling off in the amount of carbonic acid produced; such a decrease as might be assumed would naturally result from diminished proteid metabolism.

The results are shown in the accompanying tables.

The body temperature, as determined per rectum did not show any change whatever under the influence of this quantity of quinine (total dose 1·575 gram of quinine sulphate). There does not appear to be any proof that moderate doses of quinine lower the body temperature of healthy animals or even man, and Kerner found in his experiments that a full dose of quinine given to a healthy man would prevent the usual rise of temperature resulting from vigorous exercise, but did not affect the temperature under ordinary circumstances. Liebermeister* has also reported that the alkaloid has no constant depressing action on the bodily heat in health.

Two other short series of experiments were tried with quinine, also with rabbits in a condition of hunger. In both of these cases the quinine was introduced by sub-cutaneous injection in the form of sulphate. In both cases there was a slight fall in temperature, accompanied with a noticeable decrease in the amount of carbonic acid eliminated, directly after injection of the quinine. This effect, however, was only temporary, for the temperature quickly rose to the normal, and even somewhat above the normal point, while the carbonic acid in the third series came quite back to the normal and in the fourth series remained only a little way below.

It would appear, therefore, from our experiments, that in a healthy, hungry rabbit moderate doses of quinine sulphate exercise at the most only a very slight depressing influence on body temperature, and have but a minimum effect on the production of carbonic acid.

Action of cinchonidine sulphate.

Previous experiments† on man have shown that cinchonidine has the power of lessening materially the elimination of nitrogen, presumably through its inhibitory action on proteid metabolism.

Cinchonidine is supposed to have much the same physiological action as quinine and cinchonine, only weaker. Our present experi-

* Deutsch. Archiv für Klinische Medicin, Band iii.

† See Chittenden and Whitehouse, Studies from the Laboratory of Physiological Chemistry, vol. i, p. 164.

ments with the alkaloid, using quite large amounts of the sulphate, show a somewhat different action from quinine. Using a large rabbit, without food for three days, and giving it by way of the mouth, in gelatin capsules, large doses of the alkaloid, there was a very noticeable and constant rise in temperature up to the very time of death, accompanied by a slight but gradual diminution in the amount of carbonic acid given off. In all, 1·575 grams of cinchonidine sulphate were given; an amount exactly equal to the quinine sulphate given in the second series of experiments with quinine. With cinchonidine, however, the rabbit was much prostrated, showed symptoms of tetanic convulsions, and finally died in a vigorous tetanic spasm at the end of the second day.

The results are shown in the preceding table.

Action of Antipyrine.

Antipyrine or dimethyloxychinicine has of late been much experimented with. Among the many statements which we have seen recently concerning its action are the following, which are of interest in this connection. Arduin* found that 3 grams given to a rabbit produced cataleptic stiffness, diminished reflexes, etc., followed by violent convulsions. There was also a very marked fall of bodily temperature. Anseroff,† by experiments on animals, found that the alkaloid caused an increase of blood pressure and a decrease of internal temperature, as shown by a thermometer in the rectum, but a considerable rise in the external temperature, sometimes as much as 12° C. Pavlinoff‡ has reported that antipyrine produces a very considerable quickening of the respiration, while Dr. Walter, of St. Petersburg,§ is reported as having found that the alkaloid while reducing febrile temperature, also reduces nitrogenous tissue changes; and further, that the assimilation of proteids is materially favored by the drug. F. Müller‖ has also found that in fever antipyrine diminishes the excretion of nitrogen. Coppola,¶ however, states that in the case of a dog, 0·3–0·4 gram of antipyrine was wholly without action on its excretion of nitrogen. Coppola has further found that the

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* Abstract in Therapeutic Gazette, 3d series, vol. i, p. 677.

† Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 315.

‡ Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 339.

§ Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 53.

‖ Jahresbericht für Thierchemie, xiv, 242.

¶ Jahresbericht für Thierchemie, xv, 97.

FIRST SERIES OF EXPERIMENTS WITH ANTIPYRINE.

Normal period, without antipyrine.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
May 24.								
A. M.								
8:54 to 9:24	8·6	26·4	35·0	46·1	11·1	224·3	561·0	38·4
9:52 to 10:22	8·8	26·5	35·3	46·1	10·8	218·3	545·8	38·4
10:47 to 11:17	9·5	26·4	35·9	46·1	10·2	205·1	513·0	38·4
P. M.								
1:55 to 2:25	9·1	26·3	35·4	46·1	10·7	216·3	540·8	38·3
2:47 to 3:17	9·5	26·4	35·9	46·1	10·2	205·1	513·0	38·6
3:40 to 4:10	9·1	26·5	35·6	46·1	10·5	212·2	530·6	38·4
4:35 to 5:05	8·8	26·3	35·1	46·1	11·0	222·3	555·9	38·4
Average,	9·0	26·4	35·4	46·1	10·7	214·8	535·7	38·4
May 25.			*With antipyrine.*					
A. M.								
8:56 to 9:26	9·6	26·4	36·0	46·1	10·1	204·1	510·4	38·2
9:53 to 10:23	9·6	26·4	36·0	46·1	10·1	204·1	510·4	38·1
10:49 to 11:19	7·5	25·6	33·1	46·1	13·0	262·7	657·0	38·0
11:47 to 12:17	8·4	25·9	34·3	46·1	11·8	238·5	596·3	38·1
P. M.								
2:15 to 2:45	7·9	26·0	33·9	46·1	12·2	246·6	616·6	38·1
3:15 to 3:45	8·7	25·8	34·5	46·1	11·6	234·5	586·3	38·3
4:15 to 4:45	8·4	26·0	34·4	46·1	11·7	236·5	591·3	38·0
5:09 to 5:39	7·4	25·6	33·0	46·1	13·1	264·8	662·0	38·1
Average,	8·5	25·9	34·4	46·1	11·7	236·5	591·3	38·1
May 26.								
A. M.								
9:02 to 9:32	11·3	26·8	38·1	46·1	8·0	161·7	404·3	36·2
9:59 to 10:29	9·4	26·0	35·4	46·1	10·7	216·3	540·8	35·3

alkaloid not only has a noticeable antipyretic action in conditions of fever, but also reduces the temperature in healthy organisms. The extent of reduction, however, is not great, ranging only from 0·1 to 0·6 of a degree. Further, the diminution in temperature is to be ascribed, according to Coppola, not to diminished metabolic activity, but to increased giving up of heat, due to dilatation of the blood-vessels by the antipyrine. Jacubowitsch * also claims for antipyrine an inhibitory action on the excretion of uric acid. So far as our knowledge extends, however, no experiments have been tried as to the influence of this therapeutic agent on the production of carbonic acid.

Our experiments have been confined wholly to rabbits, and those in a condition of hunger. In the first series of experiments, during the antipyrine period, the alkaloid was given in large and oft-repeated doses, in the form of powder, in gelatin capsules, at follows:

May 25,	8:33 A. M.,	0·2 gram antipyrine.
" 25,	9:35 "	0·2 " "
" 25,	10:30 "	0·2 " "
" 25,	11:27 "	0·2 " "
" 25,	12:27 P. M.,	0·6 " "
" 25,	2:55 "	0·6 " "
" 25,	3:54 "	0·6 " "
" 25,	4:51 "	0·6 " "
" 25,	5:46 "	0·6 " "
" 26,	8:45 A. M.,	0·6 " "
" 26,	9:38 "	1·0 " "
		5·4

The accompanying tables show the results obtained. At the end of the last period of the second day (May 26) respiration was very rapid, about 208 per minute, and during that period the amount of carbonic acid excreted was larger than in any other. In spite of the large dose taken, however, the alkaloid appears to have had no special action on the production of carbonic acid, and further, the temperature was only very slightly lowered until on the last day, just prior to the animal's death. On the last day of the experiment (May 26) the rabbit appeared much prostrated, and at 10.35 A. M. was seized with a convulsion, followed soon after by three others, dying at 11.30 A. M.

In the second series of experiments somewhat smaller amounts of antipyrine were given, but here, as in the first series, there was no

* Jahresbericht für Thierchemie, xv, p. 444.

SECOND SERIES OF EXPERIMENTS WITH ANTIPYRINE.

Normal period, without antipyrine.

Date. May 31.	Oxalic acid to neutralize baryta solution. tube a. c. c	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
9:02 to 9:32	8·5	25·3	33·8	44.3	10·5	211·2	528·1	38·9
9:57 to 10:27	8·1	25·1	33·2	44·3	11·1	224·3	561·0	38·9
10:59 to 11:29	9·1	25·5	34·6	44·3	9·7	196·0	490·2	38·9
11:51 to 12:21	9·3	25·5	34·8	44·3	9·5	191·0	477·6	38·9
P. M.								
1:44 to 2:14	9·8	25·7	35·5	44·3	8·8	176·8	442·2	38·7
3:30 to 4:00	9·3	25·5	34·8	44·3	9·5	192·0	480·1	38·9
4:21 to 4:51	9·7	25·7	35·4	44·3	8·9	179·9	449·8	38·8
Average,	9·1	25·5	34·6	44·3	9·7	195·9	489·9	38·8
June 1.			*With antipyrine.*					
A. M.								
8:58 to 9:28	9·9	25·5	35·4	44·3	8·9	179·9	449·8	38·7
9:50 to 10:20	9·4	25·5	34·9	44·3	9·4	190·0	475·1	38·9
10:43 to 11:13	10·1	25·7	35·8	44·3	8·5	170·8	427·0	38·9
11:40 to 12:10	9·7	25·7	35·4	44·3	8·9	179·9	449·8	38·7
P. M.								
2:15 to 2:45	9·6	25·6	35·2	44·3	9·1	183·9	459·9	39·2
3:12 to 3:42	9·9	25·8	35·7	44·3	8·6	173·8	434·6	38·4
4:11 to 4:41	9·7	25·6	35·3	44·3	9·0	181·9	454·8	38·3
5:05 to 5:35	8·7	25·3	34·0	44·3	10·3	208·2	520·5	38·4
Average,	9·6	25·6	35·2	44·3	9.08	183·5	458·9	38·7

With antipyrine—continued.

Date. June 2.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a, b ard c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. °C.
A. M.								
8:56 to 9:26	10·0	25·5	35·5	44·3	8·8	177·8	444·7	38·7
9:53 to 10:23	9·8	25·6	35·4	44·3	8·9	179·9	449·8	38·6
10:47 to 11:17	8·8	25·3	34·1	44·3	10·2	206·1	515·5	37·8
11:43 to 12:13	8·6	25·1	33·7	44·3	10·6	214·2	535·7	37·1
P. M.								
2:02 to 2:32	8·6	25·1	33·7	44·3	10·6	213·2	533·2	37·1
2:55 to 3:25	8·7	25·1	33·8	44·3	10·5	212·2	530·6	37·3
3:54 to 4:24	8·8	25·3	34·1	44·3	10·2	206·1	515·5	37·6
4:47 to 5:17	8·5	25·1	33·6	44·3	10·7	216·3	540·8	37·5
Average,	8·9	25·3	34·2	44·3	10·06	203·2	508·2	37·7

Following are the average daily excretions of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with average daily body temperature:

May 31	38·8° C.	489·9 milligrams CO_2.
June 1	38·7	458·9 " "
" 2	37·7	508·2 " "

Antipyrine was given in the following quantities by mouth, in gelatin capsules:

May 31	4.57 p. m.	0·2 gram antipyrine.
June 1	8:40 a. m.	0·2 "
" 1	9:31 "	0·2 "
" 1	10:25 "	0·2 "
" 1	11:20 "	0·2 "
" 1	12:17 p. m.	0·2 "
" 1	2:52 "	0·2 "
" 1	3:49 "	0·2 "
" 1	4:48 "	0·2 "
" 1	5:40 "	0·2 "
" 2	8:45 a. m.	0·5 "
" 2	9:34 "	0·5 "
" 2	10:31 "	0·5 "
" 2	11:24 "	0·5 "
" 2	4:45 p. m.	0·2 "
		4·2

discernible action on the excretion of carbonic acid. Further, the alkaloid did not noticeably lower the body temperature until toward the close of the second day, when the quantity given had reached an amount nearly sufficient to produce a fatal result. We have then to conclude that antipyrine, at least in therapeutic doses, has no special influence on the production or elimination of carbonic acid by the rabbit.

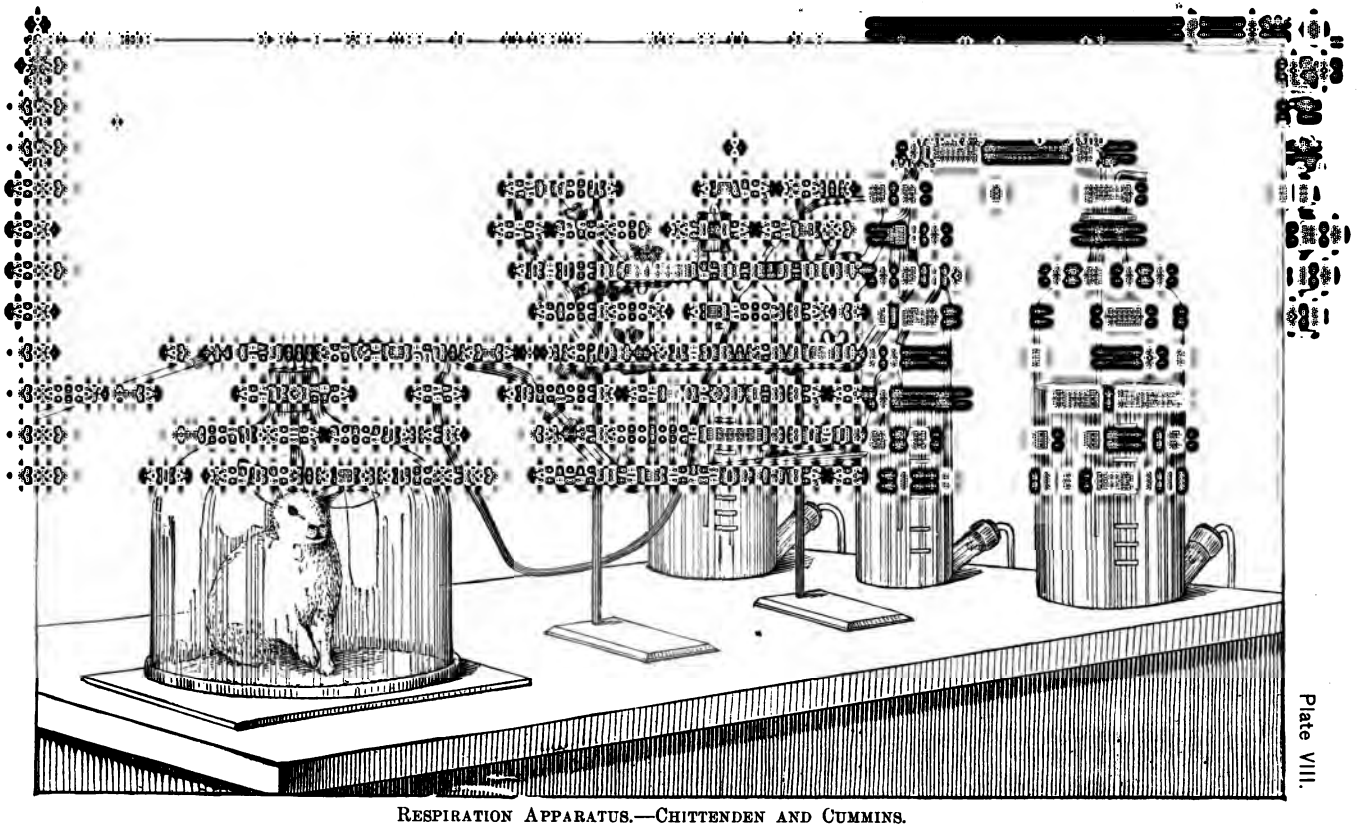

Plate VIII.

RESPIRATION APPARATUS.—CHITTENDEN AND CUMMINS.

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